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Sweep Frequency Impedance Measurements for Evaluation of Middle Ear Function in Young Infants

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ABSTRACT

Assessing outer and middle ear status in neonates and young infants is a challenging task for audiologists and medical professionals. Although standard tests such as 226-Hz tympanometry are used successfully to evaluate the function of the outer and middle ear in older children and adults, they are not diagnostically accurate for young infants less than 7 months of age (Kei & Zhao, 2012).

Sweep frequency impedance (SFI) is a new emerging technique which can provide useful information about the dynamic behaviour of the outer and middle ear in neonates and young infants. From this dynamic behaviour, the resonance frequency and mobility of the outer ear and middle ear in young infants can be measured. While there are limited case reports and pilot studies exploring the use of SFI with neonates (Murakoshi et al., 2013; Murakoshi, Zhao, & Wada, 2012), further systematic investigation in the clinical applications of SFI in neonates and young infants is essential before SFI can be used as a diagnostic tool for detecting conductive conditions in this population.

The present research study aimed to (1) establish normative SFI data for healthy Australian neonates, (2) measure the effect of ear canal static pressure on the dynamic behaviour of the outer and middle ear in healthy newborns, (3) investigate the developmental characteristics of SFI measures in infants from birth to 6 months of age, (4) compare SFI measures obtained from healthy Australian Aboriginal infants with that obtained from Caucasian infants, and (5) evaluate the test performance of SFI against individual and test battery reference standards.

Normative SFI data were developed for healthy Australian neonates (Chapter 2). The results revealed two regions of resonance, with the first resonance occurring at 287 Hz, possibly related to outer ear canal wall movement, and the second resonance occurring at 1236 Hz, possibly related to middle ear resonance.

The effect of ear canal static pressure on the dynamic behaviour of 122 ears of 86 healthy newborns and 10 ears of 10 newborns with middle ear dysfunction was studied using SFI (Chapter 3). Application of either a positive or negative static pressure to the ear canal of healthy newborns increased the resonance frequency but decreased the mobility of the outer

ear and middle ear. In contrast, in ears with middle ear dysfunction, the resonance of the middle ear was absent with no mobility of the middle ear under various static pressures. Application of negative pressure up to minus 200 daPa resulted in collapsed ear canals in more than 90% of ears.

Developmental characteristics of SFI data were obtained from 83 healthy infants from birth to 6 months using a cross-sectional study design (Chapter 4). Mean resonance frequency of the outer ear increased from 279 Hz at birth to 545 Hz at 4 months, while the mobility of the outer ear decreased with age. In comparison, the mean resonance frequency and mobility of the middle ear did not change significantly with age from birth to 6 months.

Despite Australian Aboriginal children having a higher prevalence of otitis media than Caucasian children, very few studies have compared the acoustic-mechanical properties of the outer and middle ear between Aboriginal and Caucasian neonates. SFI data from 40 ears of 24 Aboriginal neonates were compared with that from 160 ears of 119 Caucasian neonates (Chapter 5). Despite passing the test battery, Aboriginal neonates had significantly lower resonance frequencies of the outer and middle ear than Caucasian neonates. Furthermore, 22.5% of Aboriginal neonates showed no middle ear resonance, indicating the possibility of subtle conductive conditions not detected by the test battery.

The predictive accuracy of SFI in identifying conductive conditions in neonates against 4 single reference standards [automated auditory brainstem response (AABR), high frequency tympanometry (HFT), transient evoked otoacoustic emissions (TEOAE), and distortion product otoacoustic emissions (DPOAE)] and 5 test batteries standards (HFT+DPOAE, HFT+TEOAE, DPOAE+TEOAE, DPOAE+AABR and TEOAE+AABR) was evaluated (Chapter 6). The predictive accuracy of SFI was highest when measured against the HFT+DPOAE test battery reference standard, with an area under the receiving operating characteristic curve (AROC) of 0.87. The corresponding sensitivity was 86% and specificity was 88%, with positive likelihood ratio of 7 and negative likelihood ratio of 0.2. Since SFI is an accurate and valid measure of outer and middle ear function in neonates, it may be used for both screening and diagnostic assessments in neonates.

In conclusion, this thesis has not only confirmed the feasibility of testing neonates and young infants using the SFI technique, but it has also expanded the clinical application of SFI to

detecting conductive conditions in this population. While the SFI technology has shown promising results when assessing young infants, further research is needed to improve the instrumentation and test protocol for screening and diagnostic purposes.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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PUBLICATIONS DURING CANDIDATURE

Aithal, V., Kei, J., Driscoll, C., Swanston, A., Roberts, K., Murakoshi, M., & Wada, H. (2014). Normative sweep frequency impedance measures in healthy neonates. *Journal of American Academy of Audiology,* 25, 343-354. DOI:10.3766/jaaa.25.4.6

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This manuscript has been incorporated as Chapter 2 of this thesis.

Contributor	Statement of contribution
Aithal, V. (Candidate)	Designed experiments (75 %) Authored the paper (70 %) Conducted literature search (100%) Data collection (100%)
Kei, J.	Authored and edited paper (20%) Designed experiment (10%)
Swanston, A.	Authored (5%)
Katrina, Roberts.	Designed experiment (5%)
Driscoll, C.	Authored and edited paper (10%)
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STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY FOR THE AWARD OF ANOTHER DEGREE

None

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DEDICATION

To my wife Sreedevi Aithal and daughter Nimisha Aithal.
Thank you for your love and support

KEYWORDS

acoustic-mechanical properties, Australian aboriginal, conductive condition, conductive hearing loss, dynamic behaviour, infants, middle ear dysfunction, resonance frequency, sweep frequency impedance.

AUSTRALIAN AND NEW ZEALAND STANDARD RESEARCH CLASSIFICATIONS (ANZSRC)

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ANZSRC code: 110315, Otorhinolaryngology, 40%

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FoR code: 1103, Clinical Sciences, 40%

FoR code: 1114, Paediatrics and Reproductive Medicine, 40%

FoR code: 1117, Public and Health Services, 20%

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LIST OF ABBREVIATIONS USED IN THE THESIS

AABR	Automated auditory brainstem response
ABR	Auditory brainstem response
AC	Air conduction
ANHSC	Australian newborn hearing screening committee
ANOVA	Analysis of variance
AROC	Area under receiver operating curve
ASR	Acoustic stapedial reflex
ASSR	Auditory steady state evoked response
ATSI	Aboriginal and Torres Strait Islander
B	Susceptance
BC	Bone conduction
CI	Confidence interval
cm	Centimetre
daPa	Deca Pascal
dB	Decibel
DPOAEs	Distortion product otoacoustic emissions
Δ	Delta
G	Conductance
g	Gram
G-G	Greenhouse and Geisser
GR	Gradient
h	Hour
HFT	High frequency tympanometry
HHP	Healthy hearing program
HL	Hearing level
hr	Hour
Hz	Hertz
JCIH	Joint Committee on Infant Hearing
kHz	kilo Hertz
MEE	Middle ear effusion
MFT	Multifrequency tympanometry

Min	Minute
mo	Month
msec	Milli second
n	Number
nHL	Normalised hearing level
NHS	Newborn hearing screening
NHSP	Newborn hearing screening program
NICU	Neonatal intensive care unit
OAEs	Otoacoustic emissions
OM	Otitis media
OME	Otitis media with effusion
pkSPL	Peak sound pressure level
Ps	Static pressure
RF	Resonance frequency
ROC	Receiver operating characteristics
SD	Standard deviation
SEM	Standard error of mean
SFI	Sweep frequency impedance
SPL	Sound pressure level
SN	Sensorineural
SWISH	State-wide infant screening –hearing
TEOAEs	Transient evoked otoacoustic emissions
TPP	Tympanometric peak pressure
TW	Tympanometric width
UNHS	Universal newborn hearing screening
VIHSP	Victorian infant hearing screening program
WAI	Wideband acoustic immittance
WBA	Wideband absorbance
WBR	Wideband reflectance
WBT	Wideband tympanomery
wk	Week
Ya	Static admittance
Ypc	Peak compensated static admittance

Chapter 1: Introduction

1.1 Organisation of thesis

This thesis investigates sweep frequency impedance (SFI) measurements for evaluation of middle ear function in young infants. The thesis is presented as a series of published papers in international journals. The thesis consists of seven chapters each with a different focus of research. The seven chapters are described as follows:

Chapter 1 provides a review of the literature on, false positive responses in newborn hearing screening (NHS) program, external/middle ear dysfunction in newborns and young infants, overview of currently available audiological and non-audiological diagnostic tools for the assessment of outer and middle ear functions in young infants, and the background of current situation of SFI measurements, knowledge gaps and research questions.

Chapter 2 investigates the feasibility of testing neonates using the SFI technique and development of clinical norms for SFI measures in healthy neonates. This study has been published in the *Journal of American Academy of Audiology,* 25, 343-354 (2014). https://doi.org/10.3766/jaaa.25.4.6

Chapter 3 investigates effect of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns. This study has been published in the *International Journal of Pediatric Otorhinolaryngology,*82, 64-72 (2016). https://doi.org/10.1016/j.ijporl.2015.12.006

Chapter 4 investigates the developmental characteristics of sweep frequency impedance measures in infants from birth to 6 months. This study has been published in the *International Journal of Audiology,* 56, 154-163 (2016). https://doi.org/10.1080/14992027.2016.1244867

Chapter 5 investigates sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates. This study has been published in the *International Journal of Pediatric Otorhinolaryngology,* 79,1024-1029 (2015). https://doi.org/10.1016/j.ijporl.2015.04.017

Chapter 6 investigates predictive accuracy of sweep frequency impedance technology in identifying conductive conditions in newborns. This study has been published online by the Journal *of American Academy of Audiology.* https://doi.org/10.3766/jaaa.16077

Chapter 7 provides an overall discussion and conclusion based on the findings from this research.

The style of the thesis is structured according to the University of Queensland (UQ) reference style (American Psychological Association, APA 6) preferred by the School of Health and Rehabilitation Sciences. The UQ allows published journal papers to be included in the thesis. The inclusion of publications in this thesis means that different publications may contain the same or similar descriptions of concepts, test procedures and findings. For instance, the working principles of SFI had to be clearly delineated for each publication. Different referencing styles were employed as required by the journals in which the papers were published. Also, the spelling of some words might not be consistent throughout the thesis.

1.2 Introduction

1.2.1 Synopsis

Universal newborn hearing screening (UNHS) to detect permanent hearing loss using otoacoustic emissions (OAE) or automated auditory brainstem response (AABR) is becoming standard practice in Australia and internationally. Despite improvements in the screening technology, false positive referrals due to middle ear dysfunction continue to be an issue with UNHS programs. Middle ear dysfunction obliterates OAE and AABR responses, resulting in a "refer" outcome. It is, therefore, not possible to distinguish between middle ear dysfunction and cochlear hearing loss when a refer outcome occurs (Allen, Jeng, & Levitt, 2005). Several studies have reported a high rate of false positive referrals between 3 and 8% (Clemens & Davis, 2001; Clemens, Davis, & Bailey, 2000; Mason & Herrman, 1998; Mehl & Thomson, 1998; Vohr, Carty, Moore, & Letourneau, 1998). Therefore, there is a need for a tool to assess the middle ear condition and provide clinical information to differentiate between conductive and sensorineural (SN) hearing losses in infants.

Since the middle ear is involved in virtually every audiologic assessment, it is critical to ascertain the status of middle ear function to ensure correct interpretation of results. Despite promising results of high frequency tympanometry (HFT) and acoustic stapedial reflex (ASR) tests in evaluation of middle ear function in neonates and young infants, there are limitations in these tests, which may lead to erroneous test outcomes (Kei & Zhao, 2012). A new technology, sweep frequency impedance (SFI), is emerging as an alternative instrument to assess middle ear function. While the SFI has been proven to provide more accurate diagnosis of some middle ear disorders than traditional tympanometry, its use with infants has never been explored until recently. To date, there have been no published reports that have systematically investigated use of this technique with infants except for a few case reports and pilot studies (Kei & Zhao, 2012; Murakoshi et al., 2013; Murakoshi, Zhao, & Wada, 2012). For example, Murakoshi et al. (2013) noted two resonance frequency (RF) regions in ten ears of nine neonates, at 260 (low RF) and 1130 Hz (high RF), respectively. In another report, Murakoshi et al. (2012) noted that the high RF disappeared in neonates with middle ear dysfunction. This thesis seeks to investigate the feasibility of assessing the outer and middle ear function in neonates and young infants using the SFI, with a view to evaluate the test performance of the SFI in comparison with other tests of middle ear function.

1.3 Prevalence of permanent hearing loss

Permanent congenital hearing loss is one of the most common birth defects in infants (Diefendorf, 1999; Mehl & Thomson, 1998). It has been reported that significant permanent hearing loss, defined as hearing impairment of more than 40 dB HL in both ears, affects 1 to 1.5 per 1000 live births (Fortnum & Davis, 1997). However, several studies have reported an annual prevalence of 1 to 6 in every 1000 live births (Bachman & Arvedson, 1998; CDCP, 2003; Finitzo, Albright, & O'Neal, 1998). In Australia, the annual prevalence of bilateral permanent hearing loss of more than 40 dB HL is around 0.84 to 1.02 per 1000 live births (Aithal, Aithal, Kei, & Driscoll, 2012; CAHS Western Australia, 2011; Glennon, 2007; SWISH, 2011), or approximately 250 to 400 infants per year for all Australian states and territories (ANHSC, 2001; Currie, 2005).

1.3.1 Screening technology

Both OAE and AABR used for hearing screening are sensitive enough to identify cochlear hearing loss of greater than 30 dB HL (Finitzo, Albright, & O'Neal, 1998; Gorga et al., 1993; Norton et al., 2000b; Prieve et al., 1993). However, both technologies are

influenced by outer and/or middle ear dysfunction. OAE screening tests are sensitive to middle ear and cochlear conditions, while AABR can identify significant middle ear, cochlear and neural pathologies (Choo & Meinzen-Derr, 2010; Hall, Smith, & Popelka, 2004; Keefe et al., 2000). In general, OAE tends to be more affected by middle ear dysfunction than AABR (Clemens et al., 2000; Mason & Herrman, 1998; Tognola, Paglialonga, & Grandori, 2010) as both the acoustic stimulus travelling towards the cochlea and the evoked backward cochlear response are influenced by middle ear dysfunction (Margolis, 2002). Hence, with both technologies, transient middle ear dysfunction can lead to a "refer" screening test result (Doyle, Burggraaff, Fujikawa, Kim, & Macarthur, 1997). Consequently, a "refer" outcome can be the result of a conductive, SN or mixed hearing loss. In order to assess the acoustic response of the middle ear accurately and independent of the other pathologies affecting the auditory system, there is a need for an adjunct test to assess only the middle ear function in infants (Gravel et al., 2005; Keefe et al., 2000; C. A. Sanford & Feeney, 2008). The results of the assessment using this test would be useful in isolating middle ear dysfunction from cochlear dysfunction.

1.3.2 False positive responses

It is important to identify whether an infant failing the screening test has obtained a "refer" outcome because of a SN loss due to inner ear pathology or conductive loss due to middle ear dysfunction. An ear that failed the UNHS test but was later identified to have normal hearing is called a false positive response (Keefe & Feeney, 2009).

A false positive response may be caused by transient ear canal and/or middle ear dysfunction due to vernix occluding the ear canal, or residual amniotic fluid or mesenchyme in the middle ear space of well babies (Buch & Jorgensen, 1964; Keefe et al., 2000; Kok, vanZanten, & Brocaar, 1992; Rosenfeld et al., 2004; Thornton, Kimm, Kennedy, & Cafarelli-dees, 1993). Infants cared for in the neonatal intensive care units (NICU) are prone to a conductive pathology due to mechanical ventilation for long period of time (Balkany, Berman, Simmons, & Jafek, 1978; Derkay, Bluestone, Thompson, Stephenson, & Kardtzke, 1988; Paradise, 1981; L. R. Proctor & Kennady, 1990). Transient outer and middle ear dysfunction is common in infants tested within 48 hours of birth and babies in the NICU for extended periods of time (Keefe & Feeney, 2009). As most newborns are screened within the first 48 hours of birth, the temporary conductive condition may contribute to high false positive rates (Allen et al., 2005; Doyle, Rodgers, Fujikawa, & Newman, 2000).

Many UNHS programs have reported high rates of false positive responses of between 3 and 8% (Clemens & Davis, 2001; Clemens et al., 2000; Mason & Herrman, 1998; Mehl & Thomson, 1998; Vohr et al., 1998) with corresponding poor positive predictive values of 4 to 12%. With the introduction of two-stage screening programs, false positive rates have been reduced to less than 1% with subsequent increase in positive predictive value up to 24% (Clemens & Davis, 2001; Clemens et al., 2000). An analysis of Australian UNHS data for the Queensland program showed a false positive rate of 1% for all identified hearing loss with a positive predictive value of 12.3% (Glennon, 2007).

Despite the increased false positive outcomes due to middle ear dysfunction, at present, there is no single validated objective tool that can be used at the time of screening to assess middle ear function (Keefe et al., 2000). Differential diagnosis of transient conductive loss and permanent SN loss is made during follow-up diagnostic assessments which are expensive and time consuming. For this reason, Gravel et al. (2005) recommended the development of screening tools to assess middle ear function at the time of newborn hearing screening. Such tools would assist to streamline the management strategies for the respective types of hearing loss, facilitate prioritisation of infants for follow-up appointments and reduce parental anxiety. To this end, future research with multi-frequency tympanometry, sweep frequency tympanometry, acoustic reflex or wideband reflectance used as an adjunct to the AABR screening tool would be beneficial.

1.4 External/middle ear dysfunction and conductive hearing loss in newborns and young infants

The most common middle ear disorder in infants is otitis media with effusion (OME) which is defined as fluid in the middle ear cavity without signs or symptoms of acute ear infections (AAP, 2004). Acute otitis media (AOM) is the presence of middle ear effusion accompanied by signs of acute infection such as fever and irritability. As there is often a clinical continuum between AOM and OME, the term OM is generally used throughout this thesis unless specified otherwise.

1.4.1 External/middle ear pathology and conductive hearing loss in newborns

1.4.1.1 *External/middle ear pathology in newborns*

The majority of referrals in NHS programs are due to transient conductive hearing loss arising from OME and/or occluded ear canals (Doyle et al., 1997; Doyle et al., 2004; Doyle et al., 2000; Keefe et al., 2000; Kok et al., 1992; Rosenfeld et al., 2004; Takahara, Sando, Hashida, & Shibahara, 1986; Thornton et al., 1993). Several studies attribute external canal obstruction due to vernix caseosa, a waxy substance that covers the skin of the newborn, for increased false positive rates in NHS. For example, in their study of 400 ears of healthy newborns aged 5 to 48 hours, Doyle et al. (1997) found that cleaning of vernix resulted in an improvement in the ABR pass results from 91 to 96% and an improvement in the OAE pass results from 58.5 to 69%. Similarly, another study attributed 15% of failure rates to the external canal obstruction (K. W. Chang, Vohr, Norton, & Lekas, 1993). The researchers studied 82 ears of newborns with a mean age of 43 hours and found that while 76% of ears passed OAE before otoscopic examination, the pass rate improved to 91% following vernix removal.

Other studies have used otoscopic examination to determine the prevalence of vernix caseosa in the external ear canal. For instance, during otoscopic examination of 400 ears of infants aged 5 to 48 hours, Doyle et al. (1997) found vernix obscuring the view of the tympanic membrane in 53 (13%) ears. Similarly, a study of 50 infants less than 24 hours of age found that all infants had at least partial obstruction of the ear canal (Balkany et al., 1978). Another study also found that on day 1, 56% of the ear canals were obscured and this reduced to 19% by day 3 (R. M. J. Cavanaugh, 1987). Vernix can, therefore, significantly affect the outcome of NHS in the first few days of life.

Apart from vernix, amniotic fluid and residual mesenchyme have also been reported to contribute to OME and conductive hearing loss in newborns. Several temporal bone studies have shown OME to be present in up to 50% of ears (Buch & Jorgensen, 1964; deSa, 1973, 1983; Eavey, 1993). The middle ear and antrum of a newborn have been reported to contain residual mesenchyme. Studies have shown that in the early stages of foetal development, the middle ear is filled with mesenchyme, which resolves between 8 foetal months to 13 postnatal months (Guggenheim, Clements, & Schlesinger, 1956; Jaisinghani, Paparella, Schachern, & Le, 1999; Piza, Gonzalez, Northrop, & Eavey, 1989; Takahara et al., 1986).

Amniotic fluid contents aspirated into the middle ear have often been reported to contribute to OME and conductive hearing loss in newborns (deSa, 1973; Northrop, Piza, Karmody, & Eavey, 1999). The volume of amniotic fluid aspirated into the middle ear has been reported to vary significantly from a very scant amount to a large amount that fills up a substantial portion of the middle ear space (Northrop, Piza, & Eavey, 1986; Piza et al., 1989). Instead of clearing rapidly from the middle ear, the aspirated amniotic may persist for several days (deSa, 1973). Histological studies have shown that this persistent amniotic fluid material induces a significant inflammatory response of a foreign body giant cell reaction that produces a large volume of granulation tissue as well as advanced inflammatory responses. This, in turn, results in extensive damage to the major attic compartments and under pneumatization of the mastoid (deSa, 1973; Eavey, Camacho, & Northrop, 1992; Palva, Northrop, & Ramsay, 2001; Piza et al., 1989; Ramsay, Palva, & Northrop, 2001).

As a result, it is likely that during the immediate postnatal period, a conductive hearing loss may be present due to OME, followed by an improvement in hearing as this fluid is cleared (Priner, Freeman, Perez, & Sohmer, 2003). While the majority of the studies on the nature of OME in newborns have been histopathological and temporal bone studies, the prevalence of middle ear pathology and conductive hearing loss (presumably due to OME) has been derived from NHS results. For instance, Kok et al. (1992) reported the inability to record OAEs in 50% of neonatal ears 3 to 51 hours after birth, while 24 hours later OAEs could be recorded in all ears. They attributed this improvement to the clearance of fluid from the middle ear within that period. Similarly, another study attributed an ABR air bone gap of more than 12 dB within the first 48 hours after birth to residual amniotic fluid in the middle ear (Stuart, Yang, & Green, 1994). Using a combination of otoscopy, acoustic reflex measurements and tympanometry, Roberts et al. (1995) reported OME to be present in all 68 babies examined in the first three hours of life. By the third day, OME had resolved in 73% of ears by otoscopy, 88% by acoustic reflex measurements and 92% by tympanometry. Doyle et al. (1997) studied 200 newborns aged 5 to 48 hours using a combination of otoscopy, OAE and ABR and found the prevalence of OME to be 9%. Infants with decreased tympanic membrane mobility by pneumatic otoscopy had failure rates of 50% and 62.5% for ABR and OAE, respectively, compared with failure rates of 11.5% and 21% for the entire sample. Boone et al. (2005) identified OME in 64.5% of 76 infants referred for diagnostic evaluation through newborn hearing screening and attributed it to residual amniotic fluid.

In summary, vernix in the ear canal and mesenchyme or amniotic fluids in the middle ear are common causes of false positive results that affect outcomes of the NHS programs.

1.4.1.2 *Conductive hearing loss in newborns*

Many studies have reported the prevalence of conductive hearing loss due to transient middle ear dysfunction such as OME to be higher than cochlear or SN hearing loss (Aithal, Aithal, & Katrina, 2009; Aithal et al., 2012; Doyle et al., 2000; L. L. Hunter, Feeney, Miller, Jeng, & Bohning, 2010; Keefe et al., 2000; Mazlan, Kei, & Hickson, 2009; Prieve et al., 2000; Silverman, 2010; Yang, Stuart, Mencher, Mencher, & Vincer, 1993). Conductive hearing loss due to OME has been reported to be a frequent occurrence in infants attending diagnostic testing following UNHS referral (Aithal, Aithal, & Katrina, 2008; Aithal et al., 2012; Boone, Bower, & Martin, 2005; Boudewyns et al., 2011; Holster, Hoeve, Wieringa, Willis-Lorier, & de Gier, 2009; Mazlan et al., 2009). For example, in a retrospective study of 76 infants, Boone et al. (2005) attributed 64.5% of failures in NHS to OME. In another follow up study of 211 infants referred for diagnostic assessment following AABR screening, 32% had conductive loss due to middle ear dysfunction (Aithal et al., 2012). Interestingly, Mazlan et al. (2009) showed that 8.7% of 219 neonates who passed the AABR test were found to have middle ear pathology as judged by HFT and ASR results. In another study, Holster et al. (2009) reported that 20.3% of 340 neonates who failed neonatal hearing screening had conductive hearing loss related to OME. In a recent study, Boudewyns et al. (2011) reported conductive hearing loss between 40 and 60 dB HL due to OME in 84 infants aged four weeks who did not pass UNHS.

To sum up, conductive hearing loss due to external and middle ear dysfunction is a common finding in newborns that causes increased false positive rates in NHS programs. In order to reduce the false positive rates, there is a strong need to assess the conductive system at the time of screening in order to differentiate the ears with transient outer and middle ear pathologies from the ears with sensorineural hearing loss.

1.4.2 OME and conductive hearing loss in infants and young children

1.4.2.1 *OME in infants and young children*

Apart from neonates, a high prevalence of OME has also been reported in infants and young children. Studies have estimated the prevalence of OME in infants and young children to be between 15% and 40% (Casselbrant et al., 1985; Casselbrant, Mandel, Kurs-Lasky,

Rockette, & Bluestone, 1995; Paradise et al., 1997). It is reported that approximately 59% of children have at least one episode of OM by two years of age (Casselbrant, Mandel, Rockette, & Bluestone, 2003). An early childhood longitudinal study of more than 8000 children born in 2001 showed that OM was diagnosed in 39% of children by 9 months and 62% of children by 2 years of age (Hoffman, Park, & Losonczy, 2007).

1.4.2.2 *Conductive hearing loss in infants and young children*

Hearing loss due to OME can vary from 0 to 50 dB HL averaged across speech frequencies (500, 1000 and 2000 Hz) (Fria, Cantekin, & Eichler, 1985; Kokko, 1974; Wallace et al., 1988). For instance, Kokko (1974) reported a mean air conduction (AC) threshold of 27.5 dB HL and mean bone conduction (BC) threshold of 3 dB HL across speech frequencies in children. Fria, et al. (1985) measured hearing levels associated with OME in 22 infants aged 7 to 24 months and 540 children aged 2 to 12 years. They reported average speech awareness thresholds (SAT) of 24.6 dB SPL for infants and AC loss of 27 dB HL at 500, 1000 and 4000 Hz and 20 dB HL at 2000 Hz with normal BC threshold for older children. Similarly, another study reported SAT of 25.6 dB SPL for infants aged 6 to 8 months and AC threshold of 28 dB HL at 500 Hz, 27 dB HL at 1000 Hz , 21 dB at 2000 Hz and 28 dB HL at 4000 Hz for children aged 27 to 65 months with bilateral OME (Sabo, Paradise, Kurs-Lasky, & Smith, 2003). Wallace et al. (1988) prospectively studied two groups of children with confirmed OME and normal middle ear at one year of age. They noted that the OME positive group had 11 dB poorer ABR thresholds (Mean = 33.5 dB nHL) than the OME negative group (Mean = 22.3 dB nHL).

In summary, it can be seen that OME affects hearing significantly in infants and children. As OME is very common in the first few years of life, reduced hearing at this age may have a negative impact on speech and language development in affected children.

1.5 OME and conductive hearing loss in Australian newborns and young infants

OME and its sequelae are a major public health concern in Australia affecting both Aboriginal and non-Aboriginal infants and children. In 2008, the estimated cost of treating OME in Australia ranged from $100 million to $400 million (P. S. Taylor, Faeth, & Marks, 2009). Despite the high incidence of middle ear pathology and conductive hearing loss in

Australian Aboriginal infants and children, there is very limited information available in the literature on the condition of their middle ear at birth.

1.5.1 OME in Australian Aboriginal and Torres Strait Islander (ATSI) population.

The Aboriginal population comprises around 2.5% of the Australian population, but experiences the major burden of ear diseases (ABS, 2010). Torres Strait Islander people comprise 0.3% of the Australian population and 10% of the total Aboriginal population. According to the Australian Bureau of Statistics (ABS) (2010), many health and welfare outcomes of Torres Strait Islander people were similar to those for Aboriginal people. The ABS report revealed that, nationally, 9% of Aboriginal children aged 0-14 years experienced ear or hearing problems. The report also noted that more than one third (35%) experienced runny ears or glue ears (OME) and 28% experienced hearing loss or partial deafness.

1.5.2 OME and conductive hearing loss in Aboriginal and ATSI newborns, infants and young children.

1.5.2.1 *OME in young Aboriginal and ATSI infants and children*

A comparison of more than 20 published surveys conducted across Australia between 1968 and 1992 showed OME prevalence rates of between 5 and 54% among ATSI infants (Leach, 1996). Several prospective studies on Aboriginal infants in the past 30 years have shown very high prevalence of OM that varies from 50 to 100 % (Boswell, 1994; Boswell & Nienhuys, 1995, 1996; Boswell, Nienhuys, Rickards, & Mathews, 1993; Douglas & Powers, 1989; Lehmann et al., 2008; McCafferty, Lewis, Coman, & Mills, 1985; Moran, Waterford, Hollows, & Jones, 1979; Rebgetz, Trennery, Powers, & Mathews, 1989). In addition to OME, Australian Aboriginal infants and children also have a very high prevalence of tympanic membrane perforation. In her comparative study, Leach (1996) reported a prevalence rate of 14 to 53% for tympanic membrane perforations.

Prospective studies of ear examination in Aboriginal infants have shown that OM and tympanic membrane perforations are very common in the first year of life. For example, Rebgetz et al. (1989) reported that 67% of 75 Aboriginal infants examined repeatedly had a perforation in one or both ears by 12 months of age. Boswell and Nienhuys (1995, 1996) reported OM in 95% of 22 Aboriginal infants in their first eight weeks of life. Lehmann et al. (2008) reported OM in 26% of 392 examinations in non-Aboriginal children compared to

55% of 184 examinations in Aboriginal children from zero to two years of age. They reported that peak OM prevalence was 72% at five to six months of age in Aboriginal infants. It has been postulated that early bacterial colonisation with multiple bacterial types contribute to prolonged carriage and to Eustachian tube damage in Aboriginal children leading to persistent otitis media (Leach, Boswell, Asche, Nienhuys, & Mathews, 1994).

1.5.2.2 *OME in Aboriginal newborns*

Despite the high incidence of OM in young Aboriginal infants, the literature on the middle ear status of these infants at birth has been limited. Boswell (1994) studied ear status and hearing sensitivity in 41 Aboriginal infants shortly after birth and compared these with 17 non-Aboriginal infants at regular intervals over an 18 month period. In this study, Boswell assessed middle ear status using otoscopy and 226 Hz tympanometry, and hearing status using auditory brainstem response (ABR) audiometry and Visual Reinforcement Audiometry (VRA). She found that, while both Aboriginal and non-Aboriginal infants had normal hearing and middle ear function at birth, Aboriginal infants developed OM within the first eight weeks of life.

Using otoscopic and microbiological evaluation, Leach (1999) also noted that OM commenced within 3 months of age for all Aboriginal infants, progressed to chronic suppurative OM in 60% of infants and did not resolve throughout their early childhood. Lehmann et al. (2008) assessed the middle ear function of 100 Aboriginal infants from birth to two years using otoscopy and TEOAE at routine Ear Nose and Throat (ENT) clinics and found that 72% of infants had OM by five to six months of age. A recent follow up of 211 infants, using 1000 Hz tympanometry with AC and BC click ABR, referred following a UNHS program using AABR screening, showed that 32% infants had conductive loss due to OME (Aithal et al., 2012).

Collectively, there is limited literature on the middle ear status of Aboriginal infants during the newborn period. In addition, with the exception of the study by Aithal et al. (2012), all studies have used otoscopy and/or 226 Hz tympanometry to assess the middle ear condition at birth. However, both otoscopy and 226 Hz tympanometry have been found to be unreliable in the assessment of middle ear function in newborns and young infants. In view of the high prevalence of middle ear disease in Aboriginal infants and children, it is very important to document their middle ear status using the most current and appropriate

technology. Such information would be useful for providing an accurate prevalence rate of OM as well as providing appropriate management strategies.

1.5.2.3 *OME in Australian non-Aboriginal infants and children*

In contrast to the high prevalence of OME in Aboriginal infants, non-Aboriginal infants display a relatively low prevalence. For example, Boswell and Nienhuys (1995, 1996) reported that 30% of 10 non-aboriginal infants had OM in their first 8 weeks of life compared to 95% of 22 Aboriginal infants. Lehmann et al. (2008) reported OM in 26% of 392 examinations in non-Aboriginal children compared to 55% of 184 examinations in Aboriginal children from zero to two years of age. They also reported that peak OM prevalence was 40% at 10-14 months in non-Aboriginal infants compared to 72% at five to six months of age in Aboriginal infants. Moran et al. (1979) found that only 1.3% of 15,540 non-Aboriginal children had OM in one or both ears compared to 16.5% of 21,988 Aboriginal children.

In summary, Aboriginal infants have a high prevalence of OM that begins very early in life. The majority of studies that compared Aboriginal and non-Aboriginal infants have used either otoscopy and/or 226 Hz tympanometry to assess middle ear function. Research indicates that both methods are not suitable for assessing the middle ear condition in newborns and young infants. Further studies using a new technology such as SFI and wideband reflectance (WBR) to evaluate the middle ear condition of these two populations in the first year of life are required.

1.6 Developmental changes in outer and middle ear of neonates and infants

The outer and middle ear system undergoes rapid development changes from birth to six months of age (L.L. Hunter & Blankenship, 2017; Wilson, 2012). The major changes that occur postnatally are as follows.

1.6.1 Outer ear

The external ear canal is straighter and approximately 50% shorter in length in young infants (< 6 months) than in adults (Keefe, Bulen, Arehart, & Burns, 1993; McLellan & Webb, 1957; Saunders, Kaltenback, & Relkin, 1983). The infant ear canal wall has no bony portion (Anson & Donaldson, 1981), and is completely surrounded by a thin layer of elastic

cartilage at birth (McLellan & Webb, 1957), making it highly compliant, flaccid and prolapsed (Holte, Cavanaugh, & Margolis, 1990; Keefe et al., 1993; Sprague, Wiley, & Goldstein, 1985). The diameter and length of the ear canal increase from birth to 24 months of age (Keefe et al., 1993). The average ear canal diameter for a one-month-old infant is about 4.4 mm (Keefe et al., 1993) which is smaller than the 10 mm for adults (Saunders et al., 1983). The tympanic membrane is nearly horizontal relative to the external auditory canal axis in neonates, whereas it is approximately 45 degrees in adults (Anson & Donaldson, 1981; McLellan & Webb, 1957; Qi, Liu, Lutfy, Funnell, & Daniel, 2006).

1.6.2 Tympanic membrane (TM)

The tympanic membrane (TM) in newborns is thicker than that in adults. The thickness of the TM in neonates varies from 0.4 to 0.7 mm in the posterior superior quadrant, 0.1 to 0.25 mm in the posterior inferior, anterior superior and anterior inferior quadrants, and from 0.7 to 1.5 mm near the umbo (Ruah, Schachern, Zelterman, Paperella, & Yoon, 1991). The pars tensa region varies in thickness from 0.1 to 1.5 mm in newborns (Ruah et al., 1991), whereas it ranges from 0.04 to 0.12 mm in adults (Kuypers, Decraemer, & Dirckx, 2006). The tympanic ring surrounding the ear drum does not completely develop until 2 years of age (Saunders et al., 1983).

The changes in orientation and thickness of the TM occur after birth. At birth, the TM is orientated horizontally to the ear canal and gradually becomes more perpendicular to the long axis of the ear canal. At birth, the TM appears dull, whitish, and thickened. Over time, the TM thins due to loss of mesenchymal tissue (Ruah et al., 1991).

1.6.3 Middle ear

The middle ear is not completely mature at birth (Eby & Nadol, 1986; Qi et al., 2006; Saunders et al., 1983). The volume of the middle ear cavity, which includes tympanic cavity, the aditus and antrum, the mastoid antrum and mastoid air cells, in neonates is small and increases postnatally until the late teenage years. The volume of the tympanic cavity is reported to be approximately 640 mm^3 in adults and 452 mm^3 in 3-month old infants (Ikui, Sando, Haginomori, & Sudo, 2000; McLellan & Webb, 1957), and 330 mm^3 in a 22-day-old neonate (Qi, Funnell, & Daniel, 2008). The distance between the stapes footplate and ear drum in infants is shorter than that in adults. Infants have short Eustachian tubes (30mm), almost horizontal (approximately 10 degrees) and surrounded by glandular tissues (B.

Proctor, 1967). The Eustachian tube in infants opens sharply, but closes more gradually, resulting in Eustachian tube inefficiency.

The middle ear space is smaller in infants than children and adults. The length of the middle ear cavity increases during the first six months of life from the TM to the stapes footplate. At the same time, the middle ear ossicles become less dense as they absorb mesenchyme and ossify (Eby & Nadol, 1986). During this period, ossicular joints also stiffen (Saunders et al., 1983).

The middle ear of a newborn is not completely aerated. It contains amniotic fluid, exudates, mesenchyme, mucoid effusions and other materials (deSa, 1973; Palva, Northrop, & Ramsay, 1999). Aeration usually occurs during the first 48 hours, but fluid and other materials are reported to stay for a prolonged period of time in some ears. Approximately 50% of ears retain middle ear fluid by the end of the first 24 hours after birth, and it decreases to 27% after 48 hours, and 13% after 2 weeks of birth (Roberts et al., 1992).

The newborn middle ear is dominated by mass and resistance. As the child grows, the electro-mechanical properties of the outer and middle ear system are altered with increasing stiffness during the infancy period (Holte, Margolis, & Cavanaugh, 1991). As expected, these developmental changes are likely to affect the results of middle ear measures, including single-frequency and multi-frequency tympanometry. The low stiffness of the ear canal walls in neonates suggests that the external ear canal walls could move if the air pressure in the ear canal is changed. Investigators have noticed such movements during inspection of ear canals with pneumatic otoscopy (Paradise, Smith, & Bluestone, 1976). For instance, on pressurization as in tympanometry, the diameter of ear canal increased by an average of 18.3% under positive pressure or decreased by an average of 28.2% of its original value under negative pressure (Holte et al., 1990).

It is clear that the outer /middle ear system in infants is not completely mature at birth. Some structures may undergo continued development beyond six months, taking years to mature. These changes may alter the acoustic-mechanical properties and dynamics of the outer and middle ears. As traditional measures such as tympanometry and acoustic reflex tests are limited in their capacity to show developmental changes, new measures such as SFI

and wideband absorbance may shed light on how the acoustic-mechanical properties of the outer and middle ears change as the baby grows.

1.7 Overview of diagnostic tools for middle ear dysfunction due to OME in neonates and infants

Diagnostic tools for identification of OME include, otoscopy, pneumatic otoscopy, otomicroscopy and myringotomy. Traditionally the diagnosis of OME in children is based on otoscopy.

1.7.1 Otoscopy

Otoscopy is often used to diagnose OME in older children and adults, where it is possible to observe the landmarks of the ear drum such as cone of light, colour of the ear drum and handle of malleus. In contrast, the accuracy of otoscopy in the identification of OME from birth to four month-old infants has been questioned due to difficulties in observing changes in colour, mobility, reflexive reaction to light and translucency of the tympanic membrane (Jaffe, Hurtado, & Hurtado, 1970; McLellan & Webb, 1957).

There is rapid anatomical development of the outer and middle ear system in infants during the first few months of life. This includes changes in the orientation and flexibility of the ear drum and ossicular chain, rapid increase in the ear canal diameter and length (Keefe et al., 1993), formation of bony floor by 12 months of age (Kenna, 1990), and the middle ear cavity reaching adult size by 6 months of age (Eby & Nadol, 1986).

Even experienced physicians have difficulties visualising ear drums using otoscopy in newborns (Zarnoch & Balkany, 1978) due to the presence of vernix caseosa and debris in the tiny external auditory meatus (Doyle et al., 1997). Moreover, interpretation of otoscopic findings is difficult due to the less distinct landmarks of the ear drum (McLellan & Webb, 1957). Inter-observer agreement in the diagnosis of OME in newborns has been reported to vary from 27% (laRossa, Mitchell, & Cardinal, 1993) to 85% (Marchant et al., 1986). Due to large reported variations in the inter-observer agreement, Roberts et al. (1992) concluded that otoscopy cannot be relied upon in infants in the diagnosis of OME (Roberts et al., 1992).

1.7.2 Pneumatic otoscopy

Pneumatic otoscopy has been successfully used to diagnose OME in children and adults (Finitzo, Friel-Patti, Chinn, & Orval, 1992; Toner & Mains, 1990; Vaughan-Jones & Mills, 1992). However, it does not have the same success in infants due to the anatomical and physiologic changes during the newborn period (R. M. Cavanaugh, 1987) .

It has been reported that pneumatic otoscopy produces wide variations in the pressure pulses depending on whether it was introduced through a hand bulb or mouth piece (Cavanaugh, 1989). Cavanaugh (1987) performed pneumatic otoscopy on 81 healthy full term babies during the first 72 hours of life and at well baby follow-up visits. Cavanaugh reported that only 12% (14 of 115) of the ear drums visualised during the first three days of life moved briskly to pressure changes introduced by the insufflations, as compared to 44% (29 of 65) and 71% (50 of 71) of the ear drums visualised by three weeks and 10 weeks of age, respectively.

In view of the difficulties in controlling pressure and visualizing the tympanic membrane, pneumatic otoscopy has limited use in the diagnosis of OME in young infants.

1.7.3 Otomicroscopy

Otoscopy using a binocular microscope is known as otomicroscopy. It is reported to be superior than otoscopy because of significant improvement in sight due to magnification and three-dimensional vision providing depth perception (McHugh & Traynor, 2009).

Studies have shown a high correlation between myringotomy results and otomicroscopy conducted by otolaryngologists (Young, TenCate, Ahmad, & Morton, 2009). Young et al. (2009) reported a sensitivity of 94.4% and specificity of 93.8% with an overall accuracy of 94.1% for otomicroscopy when compared with myringotomy. They also noted that otomicroscopy performed on the anaesthetised child had greater diagnostic accuracy than tympanometry and pneumatic otoscopy. Similarly, studies which compared otomicroscopy with pneumatic otoscopy, tympanometry and myringotomy have found that otomicroscopy had the highest sensitivity (100%) and specificity (61.5%) among the three diagnostic tests (Lee, 2010; Lee & Yeo, 2004). Although otomicroscopy is highly accurate, it is not used regularly with young infants as it requires anaesthetisation which is not ethically justifiable.

1.7.4 Myringotomy

Myringotomy is a surgical procedure that involves making a tiny incision in the TM to relieve pressure caused by the build-up of fluid in the middle ear. Myringotomy is often used as the 'gold standard' in confirming middle ear fluid in children and adults. The main objection to this procedure is that it can only be justified in patients with specific indications such as prolonged middle ear infection or recurrent OM. In young asymptomatic infants, the use of myringotomy for research purposes is not ethically justifiable.

1.8 Overview of audiological diagnostic tools for middle ear dysfunction

It is essential to ascertain middle ear status in neonates and infants when interpreting physiological test findings. In almost all audiological assessments, the test stimulus has to pass through the middle ear before reaching the inner ear. For this reason, it is important to assess middle ear function in order to distinguish between middle ear dysfunction and cochlear dysfunction (Dhar & Hall, 2012; Margolis, 2002).

The JCIH (2007) position statement highlights the importance of monitoring middle ear status in infants. The presence of middle ear effusion can obliterate the audiologic assessment findings for infants below six months of age. In particular, the negative effect of OME on hearing is greater for infants with pre-existing SN hearing loss than for those with normal cochlear function.

1.8.1 Otoacoustic emissions (OAEs)

Otoacoustic emissions (OAEs) are acoustic emissions that are generated in the inner ear in response to short duration or transient signals. The emissions travel through the middle ear back into the ear canal where they can be measured by a miniature microphone (Kemp, 1979). OAEs can be affected or altered by external and middle ear conditions. Because OAEs require efficient transmission of sound through the outer and middle ear to and from the cochlea, the presence of normal OAEs provides some level of assurance of normal middle ear function. To date, many studies have utilised either transient evoked OAEs (TEOAEs) (Kei et al., 2003; Margolis, Bass-Ringdahl, Hanks, Holte, & Zapala, 2003; Shahnaz, Miranda, & Polka, 2008) or distortion product OAEs (DPOAEs) (C. A. Sanford et al., 2009; Swanepoel et al., 2007; Vander Werff, Prieve, & Georgantas, 2007) as reference standards to evaluate middle ear function, although these tests do not directly assess the function of the middle ear.

1.8.1.1 *Otoacoustic Emissions (OAEs) as reference standard for middle ear function*

TEOAEs or DPOAEs are often used as the "reference standard or gold standard" for normal middle ear function (L. L. Hunter et al., 2010; Kei et al., 2003; Margolis et al., 2003; C. A. Sanford et al., 2009; Shahnaz, 2008). Some researchers suggest that since both TEOAEs and DPOAEs have been found in some adult and child ears with middle ear dysfunction, passing OAEs in its strict sense cannot serve as a "gold standard" (Driscoll, Kei, & McPherson, 2001; C. A. Sanford et al., 2009; C. L. Taylor & Brooks, 2000; Thornton et al., 1993; Van Cauwenberge, Vinck, De Vel, & Dhooge, 1996). Although the presence of OAEs is not a perfect gold standard for determining the presence or absence of middle ear pathology, their measurement is dependent on an uncompromised middle ear system (Kei et al., 2003; Sutton, Gleadle, & Rowe, 1996). This reliance on normal or near normal middle ear functioning make OAEs a useful measure for providing sensitivity and specificity estimates for high frequency immittance testing in the absence of AC and BC tone ABR measurements (Swanepoel et al., 2007). As Hunter et al. (2010) suggested, OAEs provide the best comparison test available in newborns without resorting to invasive procedures, such as myringotomy, that carry risk and are not ethical in otherwise healthy newborns. Therefore, presently, TEOAEs/DPOAEs serve as a surrogate gold standard for evaluating the test performance of other measures in identifying OM in young infants.

1.9 Conventional tympanometry for children aged 7 months and over

The main advantage of the 226 Hz probe tone is its usefulness in measuring the stiffness characteristics of the middle ear system (Fowler & Shanks, 2002) in children above 7 months of age. Another advantage is that the physical volume of the ear canal (in cm^3) to admittance (in mmho) is 1:1 at 226 Hz. Hence, estimation of ear canal volume is possible when a 226 Hz probe tone is used (L. L. Hunter & Margolis, 2011).

1.9.1 Qualitative approach towards classification of tympanograms

Before the standardisation of immittance instruments, qualitative approaches were used to classify tympanograms based on arbitrary compliance units with reference to peak height and pressure, mainly based on shapes of tympanograms (Jerger, Jerger, & Mauldin, 1972; Liden, 1969; Liden, Harford, & Hallen, 1974). According to this classification system, type A tympanograms have normal peak height and location on the pressure axis, type B

tympanograms are flat with no definite peak, and type C tympanograms have a peak that occurs at a pressure less than -100 daPa. The Liden-Jerger classification also includes a type D tympanogram characterised by a double peak. Later, two subtypes, Ad and As, were added to indicate high peaked and shallow peaked tympanograms, respectively (Feldman, 1974). According to this classification system, type A is typically found in normal and otosclerotic ears, type B in ears with OME, perforated ear drum and occluded ear canal, type C in ears with negative middle ear pressure, type As in ears with ossicular chain fixation or sclerotic ears with repeated ear infections, and type Ad in ears with healed or scarred ear drum and ossicular chain discontinuity (Clark, Roeser, & Mendrygal, 2007).

1.9.2 Quantitative tympanometric measures

Following the introduction of ANSI (1987) standards, tympanograms are being classified based on quantitative tympanometric measures, which has enabled comparison of data across clinics. The four tympanometric quantitative measures are peak compensated static admittance or static admittance (Y_{tm}), tympanometric width (TW) or gradient (GR), tympanometric peak pressure (TPP) and equivalent ear canal volume (V_{eq}).

1.9.2.1 *Static admittance (Y_{tm})*

Static admittance (Y_{tm}) is a measure of the mobility of the tympanic membrane. Y_{tm} is obtained by the subtraction of ear canal admittance (which is most often measured at +200 daPa, positive tail) from peak admittance (Shanks & Shohet, 2009). Y_{tm} has been found to be useful in distinguishing conditions such as scarring of ear drum, OME and ossicular discontinuity, but not sensitive to ossicular fixation such as otosclerosis (L. L. Hunter & Margolis, 2011).

Normative values for Y_{tm} have been reported to vary depending upon the population, with infants having smaller Y_{tm} than children (Holte et al., 1991; Nozza, Bluestone, Kardtzke, & Bachman, 1992; Roush, Bryant, M., Zeisel, & Roberts, 1995). Holte et al. (1991) reported mean Y_{tm} values of 0.45 mmho for infants aged $\geq$ 4 months, while Margolis and Heller (1987) reported mean Y_{tm} values of 0.5 mmho for children aged 2.8 to 5.8 years (Margolis & Heller, 1987). As Y_{tm} values are positively skewed, some researchers report it in percentiles. For example, Roush et al. (1995) reported the 5^{th} to 95^{th} percentiles from 0.20 to 0.50 mmho for six to 12 months old, 0.20 to 0.60 mmho for 12 to 18 months old, 0.30 to 0.70 mmho for 18 to 24 months old, and 0.30 to 0.80 mmho for 24 to 30 months old children with normal

middle ear function. Kei et al. (2005) reported the 5th to 95th percentiles from 0.17 to 0.90 for children aged 5 to 6 years.

Nozza et al. (1992) reported Y_{tm} values for ears without OME and with OME who were scheduled for grommets. They reported mean Y_{tm} of 0.27 mmho (90% range = 0.10 – 0.60 mmho) for ears with OME and mean Y_{tm} of 0.73 mmho (90% range = 0.10 – 1.95 mmho) for ears without OME. In view of the significant overlap of normative values between the two conditions, the interpretation of tympanograms based on Y_{tm} needs to be interpreted along with other measures, such as tympanometric width (L. L. Hunter & Margolis, 2011).

1.9.2.2 *Tympanometric width (TW) and gradient (GR)*

Tympanometric width (TW) is a measure of the tympanogram shape in the vicinity of the peak (Fowler & Shanks, 2002). TW is taken as the width of the tympanogram at half of the height from the peak to negative or positive tail and is measured in daPa. TW denotes the sharpness of peak and has been reported to be useful in the diagnosis of middle ear pathology (Nozza et al., 1992; Paradise et al., 1976). Another measure of the sharpness of tympanogram is gradient (GR) (Brooks, 1968). Gradient is taken as the ratio of the width of the tympanogram at half of the height from the peak to negative or positive tail. The lesser the gradient means the sharper the tympanograms peak. Two studies that have compared TW and GR in children and adults have concluded that the TW is a better measure than GR in diagnosing middle ear pathology (de Jonge, 1986; Koebsell & Margolis, 1986).

Published normative values for children vary with age. Mean TW has been reported to vary from 100 daPa (90% range = 59-151 daPa) in children aged 2.8 to 5.8 years (Margolis & Heller, 1987) to 148 daPa (90% range = 102-204 daPa) in young children aged 0.5 to 2.5 years (Roush et al., 1995). A mean TW of 104 daPa (90% range = 60-168 daPa) has been reported in older children aged 3 to 16 years (Nozza et al., 1992). Kei et al. (2005) reported mean TW to be 128 daPa (90% range = 91-177 daPa) in 5- and 6-year-old children. Diagnostically, when TW exceeds the upper limit of the 90% range for infants and children, middle ear dysfunction is suspected.

1.9.2.3 *Tympanometric peak pressure (TPP)*

Tympanometric peak pressure (TPP) is the ear canal pressure at which the peak of the tympanogram occurs. It is an indicator of pressure in the middle ear space expressed in daPa.

However, the TPP may be overestimated by as much as 100% of the actual value, particularly in ears with small volume or compliant ear drums (Eliachar & Northern, 1974; Renvall & Holmquist, 1976).

To date, there has been no unanimous agreement on the interpretation of TPP in children. Negative middle ear pressure (< -100 daPa) is visually considered to be suggestive of Eustachian tube dysfunction in children. However, studies have shown that negative middle ear pressure does not always indicate Eustachian tube blockage in children. Moreover, studies have found that TPP is not a useful measure to indicate OME in children (Fiellau-Nikolajsen, 1983; Nozza, Bluestone, Kardtzke, & Bachman, 1994; Paradise et al., 1976). Hence, negative middle ear pressure is not currently recommended as a reason to refer children for treatment (ASHA, 2004). However, it is reported that children with severe negative TPP are more likely than those with normal TPP to develop OME and need to be monitored regularly (Antonio, Don, Doyle, & Alper, 2002).

At the other extreme, positive TPP has been reported in patients with acute OM (Margolis & Nelson, 1992; Ostergard & Carter, 1981) and also in ears with pinhole perforations of the ear drum (Fowler & Shanks, 2002). A positive pressure of more than 50 daPa may be suspicious of having acute OM (Margolis & Hunter, 2000). Hence, TPP may be used as a reference to assist with the diagnosis of middle ear disorders.

1.9.2.4 *Equivalent ear canal volume (V_{eq})*

Equivalent ear canal volume (V_{eq}) is an estimate of the volume of air between the probe and the middle ear when a 226 Hz probe tone is used (Lindeman & Holmquist, 1982; Shanks & Shohet, 2009; Shanks, Stelmachowicz, Beauchaine, & Schulte, 1992). The average V_{eq} is about 0.3 cm^3 in 4 months old infants (Holte et al., 1991), 0.58 cm^3 in 8 weeks to 6.7 year old children (Shanks et al., 1992), 0.75 cm^3 in preschool children aged 2.8 to 5.8 years (Margolis & Heller, 1987), 0.9 cm^3 in children aged 3 to 16 years (Nozza et al., 1992), and 0.97 cm^3 in children aged 5 to 6 years (Kei et al., 2005). Studies have shown that although normal V_{eq} does not rule out ear drum perforation, a flat tympanogram with large volume of more than 1 cm^3 suggests perforation of the tympanic membrane or patent grommet in young children (L. L. Hunter & Margolis, 2011; Shanks et al., 1992).

Measuring V_{eq} is useful in monitoring the course of middle ear disease after grommet insertion. Equivalent volume has been reported to be highly correlated to disease severity as larger volume after grommet insertion showed good prognosis (L. L. Hunter, Margolis, Daly, & Giebink, 1992). In their prospective study of 6 to 8 year old children, Hunter et al. (1992) noted that lower V_{eq} (less than 1.5 cm^3) following grommet insertion was significantly associated with greater OME recurrence. Similar findings were also reported by a large multi-centre OM study which recommended mean V_{eq} cut off for patent grommet to be greater or equal to 1.13 cm^3 (MRC, 2003).

In an attempt to demonstrate the usefulness of more than one quantitative measure in tympanometry, Nozza and colleagues (Nozza et al., 1992, 1994) compared tympanometry findings in 61 children aged 1 to 8 years with myringotomy as the gold standard. They evaluated six different protocols, three of which included ipsilateral reflex measurements. The study noted that sensitivity (90%) and specificity (86%) were highest for gradient combined with acoustic reflexes. The study also noted that gradient combined with static admittance also produced relatively high sensitivity (83%) and specificity (87%), respectively. The study also noted that positive and negative predictive values were influenced by the prevalence of disease in the population. This study also reported that test performance improved when a combination of criteria was used rather than a single criterion. For example, the use of either ipsilateral reflex or tympanometric width combined with static admittance provided better performance than using static admittance alone. In summary, the use of tympanometry in diagnostic audiology has been well established with normative data developed for children of various age brackets.

1.9.3 Conventional tympanometry in infants aged less than 6 months of age

Tympanograms recorded from normal ears of newborn infants are different from those of children older than 7 months of age. Tympanometry with a probe tone of 226 Hz has been shown to be not reliable in infants less than 6 months of age, as ears with confirmed OME have shown normal tympanograms in this population (L. L. Hunter et al., 1992; Paradise et al., 1976).

Furthermore, studies have reported flat tympanograms in some neonates with normal middle ears (Baldwin, 2006; Rhodes, Margolis, Hirsch, & Napp, 1999). For example, in a study of hearing screening in the newborn intensive care nursery, Rhodes et al. (1999) noted

that 30% of neonates who passed TEOAE screening and about 50 to 60% who passed DPOAE screening also failed 226 tympanometry, suggesting a high false positive rate for 226 tympanometry against OAEs.

According to Paradise et al. (1976), low sensitivity of the 226 Hz tympanogram is due to movement of the cartilaginous external ear canal of the infant ear with changes in air pressure, thus influencing the tympanometric shapes. However, Margolis et al. (2003) did not agree with this premise. These authors did not find an increase in admittance with negative to positive air pressure sweeps which would have provided evidence of decreased volume at negative pressure and increased volume at positive pressures changes. Nevertheless, other researchers have reported that external auditory canal distensibility in young infants does have an effect on ear canal volume measurements (Holte et al., 1990; Meyer, Jardine, & Deverson, 1997).

Complex tympanogram shapes have also been reported in infants with 226 Hz tympanometry (Kei et al., 2003; Shahnaz et al., 2008). For instance, in a study of 122 healthy full term neonates who passed TEOAE screening, Kei et al. (2003) reported that more than 51% of tympanograms were double or multi-peaked for a 226 Hz probe tone.

Given the above limitations, 226 Hz tympanometry is unsuitable for evaluating middle ear function in infants below 6 months of age. Hence, higher probe tone frequencies are recommended to assess the middle ear function in young infants.

1.9.4 High frequency tympanometry (HFT) for infants aged less than 7 months.

1.9.4.1 *660/678 Hz tympanometry*

Having acknowledged the limitations of the 226 Hz tympanometry, researchers attempted to use a higher frequency probe tone such as 660/678 Hz. Several studies have shown that 660/678 Hz probe tones are more accurate in diagnosing OME than 226 Hz probe tone in infants (Himelfarb, Popelka, & Shanon, 1979; Marchant et al., 1986; Shurin, Pelton, & Klein, 1976). Marchant et al. (1986) noted good agreement between otoscopy and 660 Hz probe tone tympanometry. Shurin et al. (1976) reported that 660 Hz probe tone tympanometry provided better separation between normal ears and ears with OME than 220 Hz probe tone tympanometry.

However, the clinical application of 660/678 Hz probe tone tympanometry has been questioned due to the presence of multi-peaked tympanograms (Himelfarb et al., 1979; Keefe et al., 1993; McKinley, Grose, & Roush, 1997). McKinely et al. (1997) evaluated middle ear function in 55 healthy newborns using a 678 Hz probe tone and reported that 18% of the multi-peaked tympanograms were classified as unusual or 'other' types. Himelfarb et al. (1979) also noted that 85% of the tympanograms recorded were multi-peaked and they attributed this to the high compliance of the external ear canal of infants. Keefe et al. (1993) measured the acoustic impedance, admittance and wideband reflectance in infants from 125 to 10,700 Hz and reported that the transmission of sound between 220 and 660 Hz into the middle ear was not efficient, due to external ear canal wall vibration and resonance, and concluded that 220 to 660 Hz is a poor frequency range to use for tympanometry with infants. They recommended that sound of frequency between 1000 and 4000 Hz where energy is most efficiently transmitted into the middle ear should be used for testing infants.

1.9.4.2 *1000 Hz tympanometry*

Having noted that many neonates who did not pass OAE/ABR screening had flat 1000 Hz tympanograms, several investigators have trialled the use of tympanometry with a probe tone of 1000 Hz for assessing middle ear function in infants (Kei et al., 2003; Margolis et al., 2003; Purdy & Williams, 2002; Rhodes et al., 1999; Sutton et al., 1996; Swanepoel et al., 2007). The 1000 Hz tympanograms tend to be single peaked in normal ears and flat in abnormal ears (Kei et al., 2003; Margolis et al., 2003; Purdy and Williams, 2002). For example, in their study of 122 newborns who passed TEOAEs, Kei et al. (2003) noted single peak tympanograms in 92% of newborns and flat tympanograms in 6% of newborns. They also noted that flat tympanograms in newborns were associated with less robust TEOAEs. Similarly, Rhodes et al. (1999) reported that 92% of 1000 Hz tympanograms were single peaked in their study and that 3 ears with flat 1000 Hz tympanograms did not pass hearing screening.

Normative admittance data for infants have been published. Kei et al. (2003) studied 122 infants aged 1 to 6 days who passed the TEOAE screening and reported that the mean Y_a compensated for ear canal effect at 200 daPa (Y_{pc}) varied from 0.39 to 2.28 mmho. The 90% range was 0.39 to 2.28 for the right ear and 0.39 to 1.95 for the left ear. Margolis et al. (2003) studied 46 ears of 30 full term babies from birth to 4 weeks of age and reported a 90% range of 0.60 to 4.3 mmho, with Y_a compensated at -400 daPa.

According to Margolis et al. (2003), the use of negative tail (-400 daPa) compensation facilitates better separation between normal and abnormal tympanograms. However, Shahnaz et al. (2008) noted ear canal collapse using the negative tail compensation method while Kei et al. (2007) noted greater difficulty in maintaining an hermetic seal at -400 daPa. Kei et al. (2007) also noted that test-retest reliability for admittance calculation was greater with positive tail compensation than with negative tail compensation.

Interpretation of 1000 Hz tympanograms has been further complicated by the presence of notched tympanograms (Shahnaz et al., 2008; Swanepoel et al., 2007). Swanepoel et al. (2007) noted that 94% of 16 ears in neonates with double peaked tympanograms passed a DPOAE screen. Therefore, they suggested that similar to single peaked tympanograms, double peaked tympanograms are also consistent with normal middle ear function in infants.

However, there is no agreement in the clinical interpretation of notched 1000 Hz tympanograms among researchers. For example, with notched tympanograms, Sutton et al. (2002) recommended the measurement of Y_a at the negative notch peak, while Margolis et al. (2003) advocated the measurement at the positive notch peak or highest peak. However, Shahnaz et al. (2008) advocated the use of the notch between the maxima for the determining Y_a in infants.

In addition to above criteria, TPP has also been used to assess the middle ear function in infants with a 1000 Hz probe tone. In a recent study, Mazlan et al. (2007) reported very large standard deviations of 45 daPa for mean TPP of 12.5 daPa at birth in a group of full term healthy neonates who had passed AABR and TEOAEs screening. The researcher also noted large deviations of 68 daPa for mean TPP of -2 daPa at 6 weeks chronological age. Similarly, large standard deviations for the mean TPP for 1000 Hz probe tone also have been reported by Swanepoel et al. (2007) for their infant populations of less than 1 week of age and 1 to 4 weeks of age. Thus, measurement of TPP does not appear to be a useful measure in tympanometric assessment of infants using a 1000 Hz probe tone (Silverman, 2010).

The 1000 Hz tympanometry offers a quick and direct measure of middle ear function in infants. According to Kei and Mazlan (2012), the advantages of 1000 Hz tympanometry

include: (1) good test performance, (2) high test-retest reliability, (3) time efficiency, and (4) availability of normative data. Using OAEs as a gold standard, the sensitivity and specificity of 1000 Hz tympanometry have been reported to vary from 0.57 to 0.91 and from 0.5 to 0.95, respectively (Margolis et al., 2003; Swanepoel et al., 2007). However, using ABR (air and bone conduction) as a gold standard, the sensitivity and specificity of 1000 Hz tympanometry applied to infants aged 2 to 21 weeks have been reported to be 0.99 and 0.89, respectively (Baldwin, 2006).

Despite the JCIH (2007) recommendation to use 1000 Hz tympanometry in infants below 7 months of age, there has been no consensus on the test protocol for infants. There is no unanimous agreement on how these tympanometric findings should be interpreted (Kei & Mazlan, 2012). As highlighted by Shanks and Shohet (2009), considerable work remains to be done before a high frequency tympanometry protocol for newborns and infants can be established. In the meantime, 1000 Hz tympanograms need to be interpreted in comparison with other measures such as OAEs, especially in infants under 7 months of age.

1.9.5 Multifrequency tympanometry (MFT)

Multifrequency tympanometry (MFT) refers to the recording of tympanograms across a wide range of probe tone frequencies which would allow the assessment of relative contribution of stiffness, mass and resistive elements of the middle ear (Colletti, 1976, 1977; Funasaka, Funai, & Kumakawa, 1984; Funasaka & Kumakawa, 1988; Shanks & Shohet, 2009). From the measurement of susceptance across the probe tone frequencies, the resonance frequency of the middle ear in adults can be estimated. There are two techniques of conducting MFT, namely, the sweep frequency and sweep pressure procedure.

Funasaka et al. (1984) developed the sweep frequency procedure for estimating resonance frequency of the middle ear transmission system, while sweep pressure procedure was introduced by Colletti (1976, 1977). At present, two commercially available admittance meters can apply both techniques. The GSI TympStar version 2 and Virtual Model 310 have test options similar to the method described by Funasaka et al. (1984), except that the measurements are done at TPP rather than at 0 daPa (Shanks & Shohet, 2009). The Virtual Model 310 also allows performing sweep pressure procedure in addition to the sweep frequency procedure. Most studies have reported estimates of resonance frequency of the middle ear in children and adults using these two instruments.

1.9.5.1 *Sweep frequency versus sweep pressure tympanometry*

Tympanograms can be obtained using either the sweep frequency or sweep pressure recording techniques. In the sweep frequency method, the probe frequency is swept from low frequency to high frequency, usually from 200 Hz to 2000 Hz. The canal pressure is then changed in discrete steps from +200 daPa to -400 daPa. Several tympanograms at frequencies of interest are recorded as the pressure is changed. In the sweep pressure method, the probe frequency is held constant while the external ear canal pressure is swept from +200 daPa to -400 daPa at a given rate (e.g., 125 daPa/sec pump speed). Multiple tympanograms at frequencies of interest are obtained.

In both methods, resonance frequency (RF) of the middle ear is defined as the frequency at which the stiffness and mass components of the middle ear admittance are equal (Margolis & Goycoolea, 1993). It is also possible to determine the RF directly from the susceptance tympanogram. Whenever the notch value on the susceptance tympanogram becomes equal to a positive tail (positive compensation) or negative tail (negative compensation), the total susceptance is zero and the system is said to be at RF (L. L. Hunter & Margolis, 1992; Shahnaz & Polka, 1997). Normal middle ear resonance in children and adults usually fall between 800 Hz to 1200 Hz. For children aged 3 to 7 years, mean RF has been reported to be 1211 Hz (800 – 1800 Hz) for sweep frequency mode and 1152 Hz (880 – 1800 Hz) for sweep pressure mode using the susceptance positive tail compensation method (L. L. Hunter & Margolis, 1992). Similarly for adults aged 18 to 56 years, mean RF was reported to be 1135 Hz (800 to 2000 Hz) for sweep frequency mode and 990 Hz (630 to 1400) for sweep pressure mode (Margolis & Goycoolea, 1993).

As newborn ears are mass dominated, resonant frequency is expected to be low. Selection of either sweep frequency or sweep pressure technique significantly affects the estimate of middle ear RF. It has been reported that a sweep frequency technique results in 105 to 183 Hz higher estimates of resonant frequency than a sweep pressure technique (Margolis & Goycoolea, 1993; Shahnaz & Polka, 1997). When a mass related pathology is suspected, use of a sweep frequency technique is recommended whereas a sweep pressure technique is recommended when a stiffness related pathology is suspected (Margolis & Goycoolea, 1993). However, most of the reported studies use a sweep frequency technique with compensation of ear canal volume at the positive tail.

1.9.5.2 *The Vanhuyse model for interpretation of MFT*

The effect of probe tone frequency on tympanometric configurations based on mathematical modelling is clearly demonstrated by the Vanhuyse model (Vanhuyse, Creten, & Van Camp, 1975). Although the Vanhuyse model was initially developed to explain the 4 normal tympanogram patterns recorded at 678 Hz, the model has also been used in accounting for changes in tympanogram shape as a function of frequency (Margolis et al., 1985). In the Vanhuyse model, different tympanometric configurations of the susceptance (B) and conductance (G) tympanograms against ear canal pressure are obtained in response to the varying probe frequencies. According to the Vanhuyse model, there are 4 types of normal tympanograms that are named on the basis of the number of positive and negative peaks and width of the peak (Vanhuyse et al., 1975; Wiley & Fowler, 1997; Wiley, Oviat, & Block, 1987). The first type of B-G tympanogram is called 1B1G because there is one peak for the B tympanogram and one peak for the G tympanogram. This 1B1G tympanogram indicates a stiffness dominated middle ear system. The other three normal variations involve notches on one or both of the tympanograms. They are 3B1G, 3B3G and 5B3G tympanograms. The 3B1G tympanogram indicates that the middle ear is less dominated by the stiffness. With the stiffness of the middle ear decreasing to zero, the middle ear begins to be dominated by mass and the probe frequency is equal to the resonance frequency of the middle ear (Zhao & Wang, 2012). The 3B3G tympanogram indicates that the middle ear is primarily mass controlled. The 5B3G tympanogram occurs when mass susceptance increases and becomes larger in magnitude than the conductance. Apart from number of peaks, the distance between the outermost peaks should be ≤ 75 daPa wide for the 3B3G tympanogram and ≤ 100 daPa for the 5B3G tympanogram, and narrower for the G tympanogram than for the B tympanogram. A B-G tympanogram is considered abnormal if it does not match any one of the 4 types of B-G tympanogram (Vanhuyse et al., 1975).

The Vanhuyse model can be used to assess the tympanograms generated using high probe tone frequencies (Holte et al., 1991; Margolis & Goycoolea, 1993). Holte et al. (1991) recorded B-G tympanograms for 23 healthy full term newborn infants using probe tones ranging from 226 to 900 Hz and track developmental changes of the tympanograms up to 4 months of age. For neonates aged 1-7 days, the tympanograms with a 226 Hz probe tone conformed to the model. But at high frequency probe tones, more tympanograms were classified as “other types”, meaning that they did not adhere to the model, and with a 900 Hz

probe tone, none of the tympanograms conformed to the model (Holte et al., 1991). Holte et al (1991) noted that, by 4 months of age, the tympanograms matched the Vanhuyse model showing that significant development had taken place.

Keefe et al. (1993) argued that low frequency probe tones are poor choice for evaluating middle ear function in newborns. They noted that the use of probe frequency from 220-660 Hz is the worst possible range to use for infants as young as 3 weeks old. They argued that the flaccidity of the ear canal walls, as well as the fluid and materials that are found in newborn middle ears, are likely to contribute to greater mass loading, resulting in more complex, notched patterns at lower frequencies seen in the Vanhuyse model. They also reported a low frequency resonance in full term infants aged 1 to 3 months, which included a region of mass-like response. Keefe et al. (1993) also modelled the low frequency admittance and reflectance responses using an oscillator model, which has its resonance at low frequencies and showed that the oscillator model represents a resonant ear canal wall motion near 450 Hz.

1.10 Emerging MFT devices

Over the years, devices for measuring middle ear function have evolved from a mechanical acoustic bridge to electroacoustic bridge, and then to computer-based systems (Zhao & Wang, 2012). Present day devices are versatile and capable of performing sophisticated measurements with the help of digital signal processing technology. Recently, new MFT techniques that measure acoustic-mechanical properties over a wide frequency range have been developed to assess outer and middle ear function. Two such techniques are Sweep frequency impedance (SFI) (Murakoshi et al., 2012; Zhao & Wang, 2012), and wideband acoustic immittance (WAI) or wideband absorbance (WBA) measures (Keefe et al., 2000; Kei, Sanford, Prieve, & Hunter, 2013; Murakoshi et al., 2012).

1.10.1 Wideband absorbance (WBA) measures

WBA measures the wideband acoustic transfer functions of the outer and middle ear from 200 to 10,000 Hz using chirps or clicks. Due to advancement in calibration and measurement techniques, WBA measures are unaffected by standing waves in the ear canal. WBA is defined as the proportion of sound energy absorbed by the middle ear. WBA varies from 1.0, meaning that all energy is absorbed by the middle ear, to 0.0, meaning that all

energy is reflected from the middle ear (Feeney & Sanford, 2012). WBA is one of the wideband acoustic immittance measures (WAI), which were designed to assess the function of the outer and middle ear. WBA measures have the potential to improve the diagnosis of middle ear dysfunction in infants, children and adults.

Studies on normative WBA have been reported in healthy neonates with normal middle ear function (Aithal, Kei, & Driscoll, 2014; Aithal, Kei, Driscoll, & Khan, 2013; L. L. Hunter et al., 2010; C. A. Sanford et al., 2009). These normative studies have shown that WBA is highest between 1000 Hz and 4000 Hz, and reduced below 1000 Hz and above 4000 Hz. The WBA absorbance at frequencies between 1000 Hz and 2500 Hz provide the best discriminability of middle ear function (Aithal et al., 2015; L. L. Hunter et al., 2010).

The WBA test has been used to detect middle ear dysfunction in newborns who failed in UNHS programs (Aithal, Kei, Driscoll, Khan, & Swanston, 2015; L. L. Hunter et al., 2010; Keefe, Zhao, Neely, Gorga, & Vohr, 2003) and conductive hearing loss in children and adults (Keefe, Sanford, Ellison, Fitzpatrick, & Gorga, 2012; Prieve, Feeney, Stenfelt, & Shahnaz, 2013). In view of its success in detecting conductive conditions in newborns, WBA has been suggested as an adjunct screening test in UNHS programs (Aithal et al., 2015; Feeney & Sanford, 2012; L.L. Hunter & Blankenship, 2017).

The WBA can be studied under both ambient and pressurised conditions, and 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 understanding of the variations in acoustic measures caused by rapid developmental changes in the outer and middle ear compared to ambient WBA measures (Aithal, Aithal, & Kei, 2017).

1.10.2 Sweep frequency impedance (SFI) technique

Initially, the SFI device was designed to measure the middle ear dynamic characteristics of adult middle ears (Wada & Kobayashi, 1990; Wada, Kobayashi, Suetake, & Tachizaki, 1989; Wada, Koike, & Kobayashi, 1998; Wada, Metoki, & Kobayashi, 1992). Its application to infants has only been explored recently (Murakoshi et al., 2013; Murakoshi et al., 2012).

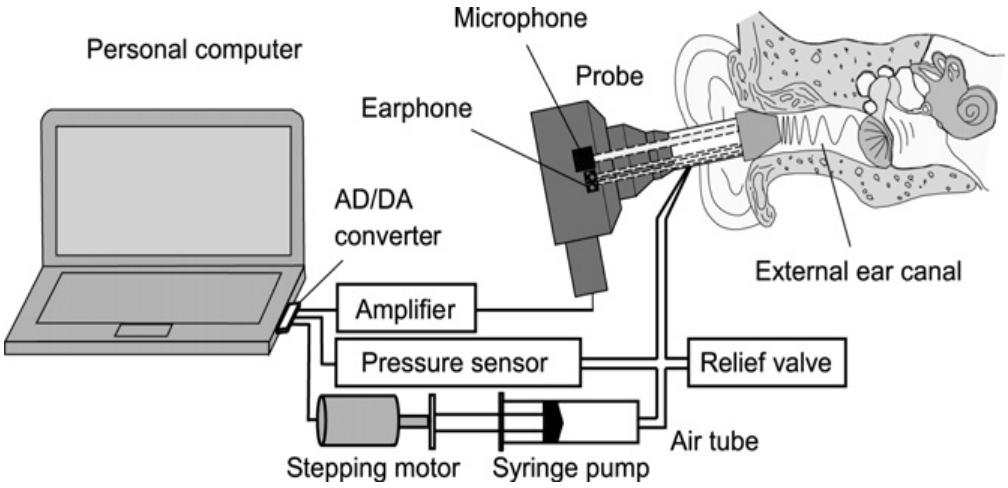

Figure 1.1. A schematic diagram of the SFI meter for testing infants. The SFI meter consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, a syringe pump, a pressure sensor and a relief valve. This new unit is controlled using LabView.

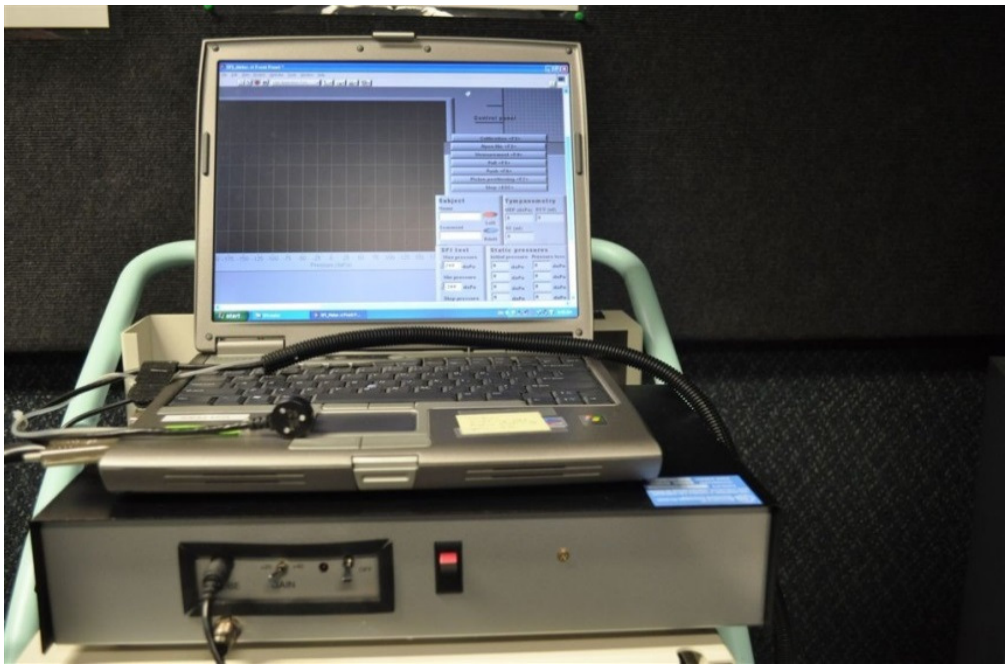

Figure 1.2. A photo of the SFI meter for testing infants

Figure 1.1 shows the schematic diagram of the SFI device which consists of a probe system, a syringe pump, a stepping motor, a pressure sensor, an AD/DA converter, pressure relief value, and a personal computer (Murakoshi et al., 2013; Murakoshi et al., 2012). Figure

1.2 shows a photograph of the actual SFI device (standing on a trolley) used for all experiments in this thesis.

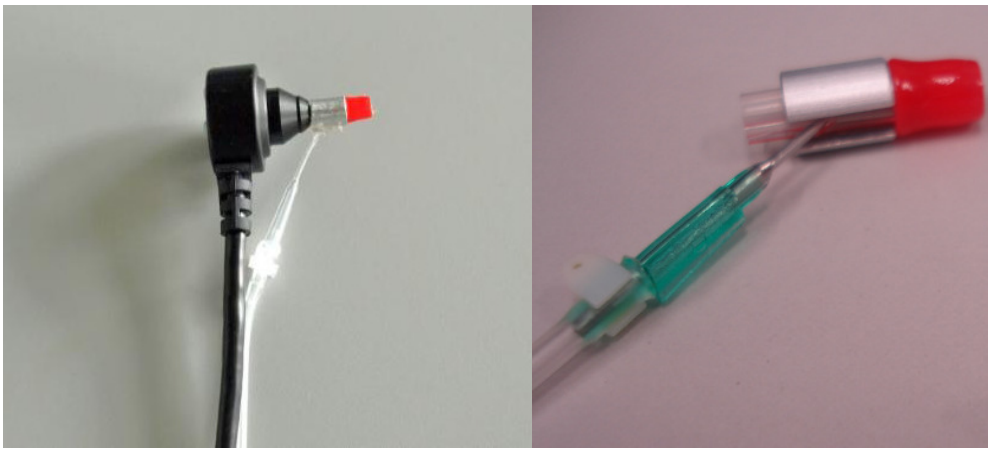

Figure 1.3. The SFI infant probes

Figure 1.3 shows photographs of the new probe system used with infants. The shape of the new infant probe is smaller than the conventional one and its diameter is approximately 3 mm whereas the conventional probe is approximately 5 mm. There are three holes in the infant probe system: one for delivering sound to the external auditory canal, one for applying static pressure, and the remaining one for measuring sound pressure using a microphone. A specially designed cuff suitable for testing an infant's ear is attached to the tip of the probe to obtain a hermetic seal of the ear canal during testing. The probe used in the SFI meter has been designed to have a flat frequency characteristic over the frequency range from 100 to 2000 Hz (Murakoshi et al., 2012; Wada et al., 1989). Hence, this probe measures sound pressure levels (in dB SPL) against frequency (in Hz) and static pressure (in daPa) in this frequency range.

During the test, the probe tone frequency is swept from 100 Hz to 2200 Hz while the external auditory canal static pressure is held constant at a predetermined level. The measurements are performed at 50 daPa intervals from 200 daPa to – 200 daPa and also at tympanometric peak pressure point of the conventional tympanogram where the peak occurs (Wada & Kobayashi, 1990; Wada et al., 1989). The SFI meter also plots admittance tympanograms, such as 1000 Hz tympanograms for infants or 226 Hz tympanograms for children above 6 months before sweeping the frequencies. The probe tone level is maintained at or below 75 dB SPL to avoid eliciting a stapedial muscle reflex response. The sweeping

tone is calibrated using a 2-cc coupler at 1000 Hz. The entire procedure for the automatic recording of results takes less than 1 minute per ear. In addition to this, the SFI unit has an inbuilt mechanical pressure relief valve as well as provision to measure loss of pressure before and after the sweep.

The SFI technique utilizes a different approach from the conventional MFT. Unlike the MFT, the SFI does not measure the admittance of the outer and middle ear. Instead, it measures the sound pressure level (SPL) in the ear canal while stimulus frequency is swept from 100 to 2200 Hz at various static applied air pressures. Using a different measurement method to the MFT (Colletti, 1977; Funasaka et al., 1984), the SFI meter measures the RF of the middle ear and the mobility of the ear drum in terms of changes in sound pressure level (Δ SPL). This adds additional dimensions to measuring the dynamic properties of the middle ear. According to Wada et al. (1989, 1990), dynamic behaviour of the middle ear refers to the measurement of RF and volume displacement of the ear drum (Δ SPL).

RF is the frequency of the sound which travels through the middle ear system with minimal resistance. The RF measured using the SFI device refers to the RF of the auditory system consisting of the ear drum, the ossicular chain and the middle ear air cavities (Wada et al., 1989). When testing a healthy adult, the change in sound pressure level (Δ SPL) in the ear canal varies considerably at the frequency between 800 to 1200 Hz at a static ear canal pressure of 0 daPa. The RF of the middle ear is determined within this frequency range (Wada & Kobayashi, 1990; Wada et al., 1989). As the air pressure in the external auditory canal increases, the RF increases while the Δ SPL decreases. It is also important to note that the RF of the middle ear is not much affected by the external auditory canal volume (Wada et al., 1989).

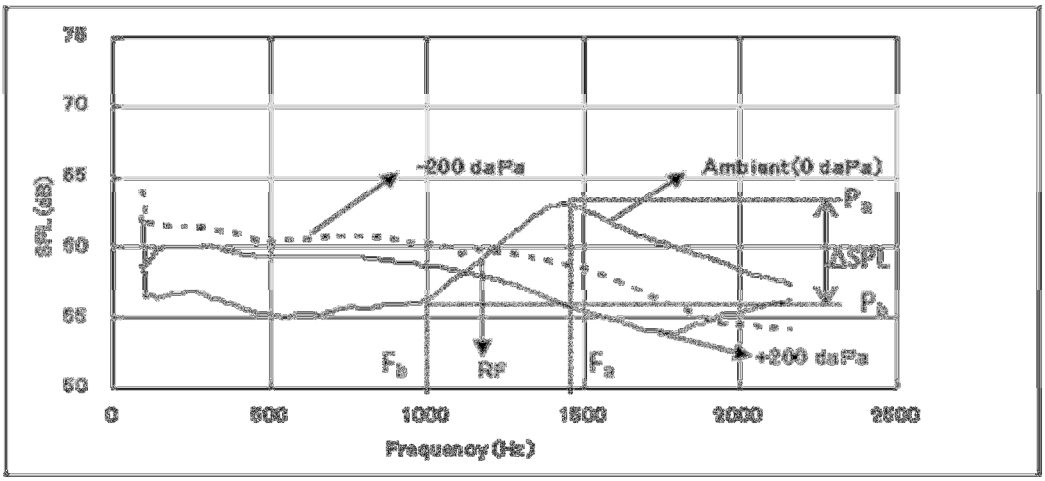

Figure 1.4. An example of SFI tracings obtained from a normal hearing adult who passed 226-Hz tympanometry.

The unique features of the SFI test are that the RF and middle ear mobility in terms of sound pressure change (Δ SPL) can be measured directly from the results. Figure 1.4 shows the SFI tracings obtained from a normal hearing healthy adult who passed 226 Hz tympanometry. The SPL curve corresponding to the ambient ear canal static pressure of 0 daPa shows a region of rapidly increasing pressure with the maximum and minimum sound pressures indicated by P_a and P_b, respectively, and frequencies corresponding to these sound pressures at F_a and F_b, respectively. Once these frequency and sound pressure points are determined, both RF and Δ SPL can be calculated using the following equations:

Resonance frequency (RF) = $(F_a + F_b)/2$

Ear drum mobility (Δ SPL) = $P_a - P_b$

As shown in the Figure 1.4, when static pressure (Ps) = 0 daPa (ambient pressure), RF = 1220 Hz, and ΔSPL = 8 dB. In ears with middle ear dysfunction, the middle ear mechanics are altered, resulting in significant changes in RF and Δ SPL. Hence, SFI has a great potential to detect middle ear dysfunction with high accuracy through the different mechanism of measurement of RF and mobility of the middle ear. However, these unique features of the SFI technique have not yet been explored in infants.

1.10.2.1 *Clinical applications in adults*

1.10.2.1.1 *Normative data in adults*

For the first time, Wada et al. (1989, 1990) reported normative data for RF and Δ SPL for 50 normal adults using a SFI meter. They noted that the Δ SPL curve varied on a large scale (≈ 5 dB SPL) around 0.8 to 1 kHz and considered this range as the RF region of the middle ear. They found that the RF region increased and the Δ SPL decreased with an increase in external auditory canal air pressure. The Δ SPL decreased monotonously with increase in frequency when the air pressure in the external canal was equal to 100 daPa. This meant that the ear drum vibration was almost suppressed when the air pressure introduced into the external auditory canal exceeded 100 daPa. They attributed these changes to the variation of ossicular chain angular stiffness which increases with an increase in air pressure in the external auditory ear canal (Wada & Kobayashi, 1990).

Wada et al. (1998) also reported normative data for middle ear dynamic characteristics in 275 ears of adults with intact ear drums and normal hearing. They reported a mean RF of 1.17 kHz (SD: 0.27) and mean Δ SPL of 2.18 dB SPL (SD: 3.84 dB).

1.10.2.1.2 ***Sweep frequency impedance findings in ears with middle ear dysfunction***

There are very few studies that have compared SFI with MFT or that have used the SFI technique in assessment and diagnosis of middle ear dysfunction such as ossicular chain disorders, OM and ear drum perforation (Wada & Kobayashi, 1990; Wada et al., 1989; Wada, Kobayashi, & Tachizaki, 1992; Wada et al., 1998; Zhao et al., 2002). Wada et al. (1989, 1990) noted that in the case of ossicular chain discontinuity due to separation of incudostapedial joint, the RF was lower than the normal value. They found the RF to be about 660 Hz at 0 daPa ear canal pressure and Δ SPL about 18 dB SPL, which was more than three times that of the normal values (Wada et al., 1989; Wada et al., 1998). They also noted an increase in RF and decrease in Δ SPL with increasing static pressure difference between the ear canal and tympanic cavity (middle ear) in ears with ossicular chain discontinuity. However, the rate of RF increase and Δ SPL decrease was smaller than that of normal hearing subjects. Moreover, Wada and Kobayashi (1990) observed the RF to be around 1500 to 1700 Hz when the static pressure in the ear canal was +200 daPa for ossicular chain discontinuity subjects and this variation was not observed in normal hearing subjects.

In contrast, in the case of a mild ossicular chain fixation such as otosclerosis where tympanometry showed Type A tympanograms (Jerger, 1970), the RF region has been reported to shift to a higher frequency range (1800 Hz), with smaller Δ SPL values (less than

2 dB SPL) than in normal subjects (Wada et al., 1989). The RF disappeared with slight increase in static pressure in the external auditory meatus (Wada et al., 1998).

In the case of a severe ossicular chain fixation where tympanometry showed type B tympanograms (Jerger, 1970), irrespective of the application of static pressure, the RF region was not observed between 100 Hz to 2000 Hz (Wada et al., 1989). Furthermore, Zhao et al. (2002) reported that the SFI test identified three distinct categories of middle ear dynamic characteristics based on RF in otosclerotic patients, such as high stiffness, normal stiffness and low stiffness middle ear status. They reported that these types of middle ear dynamic characteristics are most likely related to the different stages of the pathological changes which are difficult to identify using conventional 226 Hz tympanometry or MFT.

Wada et al. (1992) reported the effect of diameter or size of eardrum perforation and size of mastoid cavity associated with perforated ear drums on middle ear dynamic characteristics. RF and ΔSPL have been reported to vary with the cause of perforation and intactness of the mastoid cavity. Wada et al. (1992) also reported that RF decreased considerably with increase in mastoid cavity volume. The effect of mastoid cavity volume on Δ SPL variation at the RF was small. In the case of traumatic perforation with a well pneumatised mastoid cavity, the RF is reduced in the vicinity of 350 Hz, with doubling of Δ SPL. In contrast, in the case of perforation due to chronic OM, the RF increased above 1000 Hz to the vicinity of 1800 Hz, and the Δ SPL curve showed minimum values. Wada et al. (1992) also noted that in the case of perforation along with attic and/or aditus and antrum granulation and/or mucosal hypertrophy, the RF were distributed between 1100 and 2000 Hz and Δ SPL had minimum values. When no such pathology was found, minimum Δ SPL curves were distributed between 230 and 630 Hz (Wada, Kobayashi, et al., 1992). It is important to note that these types of differential diagnosis are not possible in adults while using 226 Hz tympanometry or MFT.

In the case of OME with type B tympanogram (Jerger, 1970), the RF was not observed. In case of type C tympanogram (Jerger, 1970), the results were similar to those obtained with normal hearing subjects except for the value of the static pressure where the largest Δ SPL was noted. Further, the value of Δ SPL was largest when the pressure in the external auditory meatus was equal to TPP (Wada et al., 1998).

In the case of atelectatic ear drum, the middle ear behaved like ossicular chain discontinuity with lower RF and larger Δ SPL than that of normal values. The effects of external auditory canal static pressure on middle ear dynamics were smaller than those of a normal hearing subject (Wada et al., 1998).

While there are no large scale studies on SFI, limited studies done on a variety of middle ear pathologies show that this technology holds promise for further investigation into middle ear functioning in various age groups. The ability of SFI to measure the RF and Δ SPL in ears with type B tympanograms is a distinct advantage over MFT. The studies also reveal that SFI can measure subtle middle ear disorders, whereas it is difficult to identify such disorders using conventional 226 tympanometry and MFT. However, there are no large scale studies which report the benefit of identifying such subtle changes in the differential diagnosis of middle ear disorders.

1.10.2.2 *Clinical applications in infants*

Although the SFI technique has been used successfully in adults to identify middle ear dysfunctions, its application to infants and children has never been formally explored until recently. Figure 1.4 and 1.5 show SFI results obtained from a healthy normally hearing adult and infant, respectively.

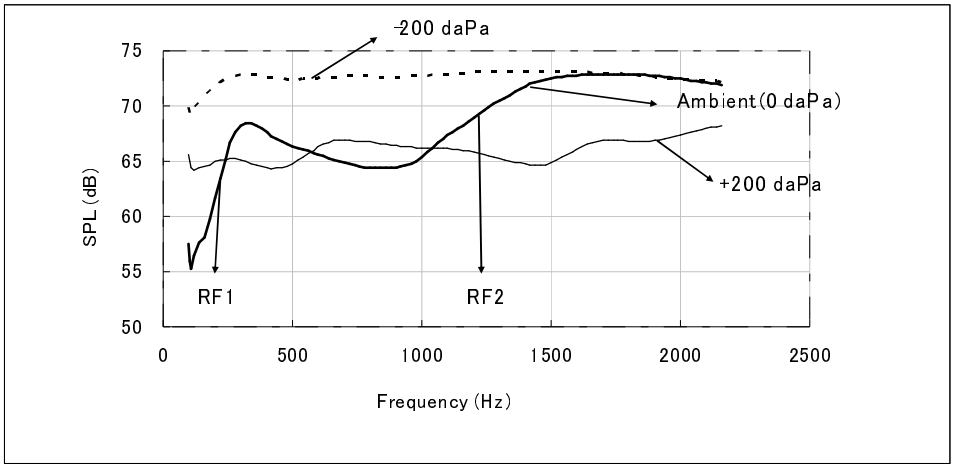

Figure 1.5. SFI results obtained from a healthy normal hearing one-day-old infant (study participant) who passed HFT and TEOAE. The SPL curve at ambient pressure shows two variations in sound pressure, the first (RF1) at around 260 Hz and the second (RF2) at around 1220 Hz. Note: RF=resonance frequency

As shown in Figure 1.4, the SPL for the adult varied greatly at the frequency around 1220 Hz, being the RF of the middle ear at a static pressure (Ps) of 0 daPa. Interestingly, SFI results for the infant (Figure 1.5) did not resemble the results for the normal hearing healthy adult. In the infant case, when the static pressure in the external auditory canal Ps was 0 daPa (ambient pressure), the Δ SPL varied greatly at around 260 Hz (RF1) and 1220 Hz (RF2).

To date, there have only been two studies that have used the SFI technique in the measurement of RF and Δ SPL of the middle ear in infants (Kei, Mazlan, Seshimo, & Wada, 2010; Murakoshi et al., 2013). In one study, Kei et al. (2010) obtained SFI results from 24 normal neonates, aged 1 to 4 days, and recorded mean RF of 287 Hz (SD=35 Hz) and mean Δ SPL of 8.8 dB (SD=2.8 dB). Two newborn babies, aged 2 days, with OM showed RF less than 200 Hz and Δ SPL of less than 3.2 dB. This study also reported an upper RF at around 1233 Hz and Δ SPL of 4.8 dB. A similar study conducted by Murakoshi et al. (2013) on healthy infants showed a lower RF region at 260 Hz with mean Δ SPL1 of 9.3 dB SPL, and an upper RF region at 1130 Hz with mean Δ SPL of 3.6 dB.

As seen in these two studies, healthy neonates showed two resonances in the outer and middle ear system. Murakoshi et al. (2013) found two vibrating elements in the neonatal external and middle ear corresponding to two variations in the Δ SPL observed at 300 and 1200 Hz. According to Murakoshi et al. (2013), the variation in Δ SPL which occurs around 1200 Hz may be related to the RF of the middle ear, whereas the other variation in Δ SPL which occurs around 300 Hz may be associated with the infant ear canal being more elastic than that of the adult.

1.10.3 Comparison of SFI and MFT in young infants

More than one RF has been reported with SFI in infants (Kei et al., 2010; Murakoshi et al., 2013; Murakoshi et al., 2012). Similar to SFI, several studies have reported more than one RF while using MFT. Holte et al. (1991) reported that in infants less than one month of age, RF was noted at about 450 Hz and another at about 710 Hz. They also observed that in their older group, the lower RF disappeared and the higher RF increased to more than 900 Hz. Although more than one RF is seen while performing MFT using GSI 33 Tympstar, this instrument automatically defaults to a lower RF (Valvik, Johnsen, & Laukli, 1994). Even though studies have reported more than one RF of the middle ear (Hocke et al., 2000; Shanks, Wilson, & Cambron, 1993), their statistical evaluation has been limited to determination of

the main RF. At present, analysis of all of the RF is not performed and, to some extent, this is due to the limited information provided by the MFT patterns as a result of low frequency resolution (Hocke et al., 2000).

It has also been reported that movement of the ear canal in infants due to the canal not functioning as a hard wall cavity makes it difficult to separate the contribution of ear canal resonance from the middle ear resonance (Sprague et al., 1985) when using MFT in infants. However, studies have indicated that the RF region of the middle ear is less affected by the external auditory canal volume when using the SFI technique (Wada et al., 1989). Other studies support the notion that middle ear resonances are not dependant on the ear canal volume (Hocke et al., 2000).

Despite the advantages of SFI over conventional 226 Hz tympanometry and MFT, there are no studies which measure the sensitivity and specificity of SFI in diagnosing different middle ear pathologies except for pathologies involving the ossicular chain (Wada et al., 1998; Zhao et al., 2002). Zhao et al. (2002) noted that high RF and low Δ SPL were associated with abnormally high stiffness of the middle ear system in the case of otosclerosis and significantly a higher percentage of abnormal stiffness was noted while using the SFI test compared with 226 Hz tympanometry in adults. Wada et al. (1998) noted that although there is an overlap of RF in normal and otosclerotic ears, the SFI separates the region of ossicular fixation from that of ossicular discontinuity (separation) better than that of 226 Hz tympanometry in adults.

Although there have not been many studies conducted with infants using SFI, Kei and Zhao (2012) have summarised the SFI in infants suggesting that, (1) Δ SPL is not independent of RF, (2) there is an overlap of RF and Δ SPL between individuals with normal middle ear function and those with middle ear disorders, (3) the origin of the vibrating element that occurs around 300 Hz in infants is still unclear, and (4) the variation of the Δ SPL curve located at 1200 Hz in infants covers a wide frequency range and the inflection of the SPL curve is not as distinct as that for adults. At present, the SFI device has not been commercialized. To utilize SFI as a clinical device with infants and young children, further research is needed to develop clinical norms in healthy infants and compare them with infants with middle ear disorders.

1.11 Rationale for the study

With the introduction of UNHS nation-wide, there is a need to address the false positive referrals from these programs, as the majority of these referrals are caused by middle ear dysfunction (Clemens & Davis, 2001; Clemens et al., 2000; Doyle et al., 2000; Keefe et al., 2000; Mehl & Thomson, 1998; Vohr et al., 1998). Additionally, middle ear problems in infants with SN hearing loss will further complicate and delay diagnosis and intervention (Boone et al., 2005). Analysis of UNHS referrals for diagnostic assessments in Northern Queensland showed that the prevalence of conductive hearing loss due to OME was higher than that of permanent SN hearing loss (Aithal et al., 2012). This study also revealed that 32% of infants referred for diagnostic assessments had conductive loss due to middle ear dysfunction. Hence, there is a need for a reliable and objective measure of middle ear function at the time of screening. While the SFI is useful in identifying middle ear disorders in adults, its application to infants has not been systematically studied.

1.11.1. Justification for conducting the present study

A review of literature has identified several issues regarding middle ear assessment in neonates and young infants. The present study seeks to address five of these issues.

First, assessment of middle ear function in infants up to 6 months of age is a challenging task. Although HFT is recommended for assessment of middle ear function in young infants (JCIH, 2007), there are no standardized test protocols and no universally agreed methods for interpreting HFT results (Kei & Zhao, 2012). This calls for trialling new technologies in the assessment of middle ear function. Although SFI measurements have been successfully used to diagnose different middle ear disorders in adults (Wada & Kobayashi, 1990; Wada et al., 1989; Wada, Kobayashi, et al., 1992; Wada et al., 1998; Zhao et al., 2002), its application to infants has never been explored fully except for a few preliminary studies (Murakoshi et al., 2013; Murakoshi et al., 2012). With this technique, it is possible to measure the resonance frequency and mobility of the middle ear which may have diagnostic significance in differentiating middle ear dysfunction from normal middle ear function in infants and this is a wholly novel aspect. As a prospective clinical tool, SFI needs to be evaluated in infants with normal middle ear function and middle ear dysfunction. For the first time, the present study seeks to apply SFI in the assessment of middle ear function in neonates on a large scale. There is very limited information on normative SFI data in

newborns (Kei & Zhao, 2012; Murakoshi et al., 2013). If SFI is to be considered as a useful measure in evaluating middle ear function in infants, the development of normative data is crucial. The present study will describe the dynamic properties of the outer and middle ear in healthy Australian neonates, with a view to developing normative SFI data for healthy newborns who have passed HFT, TEOAE and AABR.

Second, the outer and middle ear system in newborns are not mature at birth. The dynamic behaviour (acoustic-mechanical properties) of the outer and middle ear is altered when an external air pressure is applied to the ear canal, as in tympanometry. The changes in dynamic behaviour of the outer and middle ear need to be investigated using the SFI technique in newborns with and without a conductive condition if SFI is to be used as a mass screening and diagnostic tool in UNHS programs. This study will offer useful clinical information for differentiating healthy ears from ears with conductive conditions in newborns.

Third, for SFI to be used with infants beyond the newborn period, appropriate age dependent norms need to be developed. Due to rapid anatomical and physiological changes of the outer and middle ear in the first few months of life, the SFI results will vary, depending on the stage of development of the ear. Hence, age dependant norms need to be established for accurate assessment of middle ear function. Apart from the data obtained from a few preliminary pilot studies with newborns (Murakoshi et al., 2013; Murakoshi et al., 2012), there have been no age dependant SFI norms published for young infants. The present study will measure SFI in infants at 0, 1, 2, 4 and 6 months of age to track developmental changes and develop norms for infants in their first 6 months of life.

Fourth, despite the high prevalence of middle ear diseases in Australian Aboriginal infants and young children (Douglas & Powers, 1989; Foreman, Boswell, & Mathews, 1992; Leach, 1999; Morris, Leach, & Silberberg, 2005), there is a lack of research in evaluating outer and middle ear function in this population using newer technologies. Boswell and colleagues have utilised otoscopy and 226 Hz tympanometry in the assessment of middle ear function in newborns (Boswell, 1994; Boswell & Nienhuys, 1995, 1996; Boswell et al., 1993). However, both these measures are not reliable in newborns and young infants. There are no reported studies of assessment of middle ear function in Aboriginal newborns using HFT, ASR or SFI. Evaluation of middle ear status at birth using these technologies in both

Aboriginal and non-Aboriginal infants is essential. The present study will measure outer and middle ear function using the SFI technology with both Australian Aboriginal and Caucasian neonates. By comparing the SFI results between the two groups, this study will provide valuable clinical information on the function of the conductive pathway (outer and middle ear) for the two ethnic groups at birth.

Last, if SFI is to be used as a measure of middle ear function, it needs to be assessed against a strict gold standard. Studies have often considered using "passing the DPOAE or TEOAE test" as a reference standard for normal middle ear function (L. L. Hunter et al., 2010; C. A. Sanford et al., 2009). However, a single measure such as TEOAE or DPOAE may not identify subtle middle ear pathologies (Driscoll et al., 2001; Kei et al., 2003; Kemper & Downs, 2000) and, hence, may not be an ideal reference standard (L. L. Hunter et al., 2010; C. A. Sanford et al., 2009). Instead, a battery of tests would provide a robust measure of middle ear function. To date, there have been no studies that measure SFI against a battery of tests as a reference standard for middle ear function. The present study will evaluate the predictive accuracy of SFI in terms of its ability to identify conductive conditions in neonates when compared with 9 different reference standards. These standards consist of single tests and composite test batteries including HFT, AABR, TEOAE and DPOAE. The study will evaluate whether the SFI can provide an accurate measure of outer and middle ear function in neonates.

1.12 Aims of the thesis

The aims of the thesis are:

(1) To investigate the feasibility of testing neonates using the SFI technique, describe the dynamic behaviour of the outer and middle ear in healthy neonates who passed a battery of tests including AABR, TEOAE and HFT, and establish normative SFI data for resonance frequency (RF) and mobility of the outer and middle ear in terms of changes in sound pressure level (Δ SPL in dB) (see Chapter 2).

(2) To measure the effect of ear canal static pressure on the dynamic behaviour of the outer and middle ear in healthy newborns (see Chapter 3).

(3) To conduct a cross-sectional study to determine the developmental characteristics of SFI measures on a sample of healthy young infants aged 0, 1, 2, 4 and 6 months (see Chapter 4).

(4) To compare SFI measures obtained from healthy newborn Australian Aboriginal infants with those obtained from Caucasian infants (see Chapter 5).

(5) To evaluate the predictive accuracy of SFI in terms of its ability to identify conductive conditions in neonates when compared with nine different reference standards consisting of single tests and composite test batteries including HFT, AABR, TEOAE and DPOAE (see Chapter 6).

To achieve these aims, the following studies were carried out:

(1) "Normative sweep frequency impedance measures in healthy neonates" (Chapter 2).

(2) "Effect of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns" (Chapter 3).

(3) "Sweep frequency impedance measures in young infants: Developmental characteristics from birth to 6 months" (Chapter 4).

(4) "Sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates" (Chapter 5).

(5) "Predictive accuracy of sweep frequency impedance technology in identifying conductive conditions in newborns" (Chapter 6).

1.13 Hypotheses of the Study

This thesis contains 4 null hypotheses to be tested. They are

H_0 1: There is no significant difference in mean values of SFI measures (RF and Δ SPL) of the outer and middle ear when the static ear canal pressure is changed from +200 to -200 daPa in newborns with and without conductive conditions.

H_0 2: There is no significant age effect on SFI measures obtained from infants aged 0 (birth) to 6 months.

H_0 3: There is no significant difference in SFI measures between Australian Aboriginal and Caucasian neonates.

H_0 4: There is no significant difference in the predictive accuracy of SFI between single tests and test battery reference standards.

Chapter 2: Normative Sweep Frequency Impedance measures in healthy neonates

2.1 Background

Whereas Sweep Frequency Impedance (SFI) has been reported to be useful in the diagnosis of various middle ear conditions in children and adults, its application to evaluating outer and middle ear function in neonates has not been investigated thoroughly. As the original research prototype developed by Wada et al (1989) for use with adults was not suitable for use with neonates, a modified version of the SFI unit was developed by Murakoshi et al (2013) and used in the present study. This new unit has a small probe suitable for young infants (< 7 months of age). The validity and calibration of this unit for testing young infants have been confirmed by Murakoshi et al (2013).

To date, no large studies have yet systematically investigated the use of SFI with neonates. This paper investigates the feasibility and usefulness of SFI measures for evaluating middle ear function in healthy Australian neonates. The study also provides normative SFI data obtained from a prospective sample of 100 healthy neonates who passed a battery of tests that included AABR, TEOAE and HFT.

Chapter 2 of this thesis, entitled "Normative sweep frequency impedance measures in healthy neonates" is based on the manuscript published in the *Journal of the American Academy of Audiology*. The paper is inserted into this thesis with minor modifications. Only the formatting of section sub-headings and numbering of tables and figures have been modified from the original publication to match the thesis format. The referencing format of the paper is retained as per the journal format.

Aithal, V., Kei, J., Driscoll, C., Swanston, A., Roberts, K., Murakoshi, M., & Wada, H. (2014). Normative Sweep Frequency Impedance measures in healthy neonates. *Journal of the American Academy of Audiology,* 25, 353-354. DOI:10.3766/jaaa.25.4.6

2.2 Abstract

Background: Diagnosing middle ear disorders in neonates is a challenging task for both audiologists and otolaryngologists. Although high frequency (1000-Hz) tympanometry and acoustic stapedial reflex tests are useful in diagnosing middle ear problems in this age group, they do not provide information about the dynamics of the middle ear in terms of its resonance frequency and mobility. The sweep frequency impedance (SFI) test can provide this information which may assist in the diagnosis of middle ear dysfunction in neonates.

Purpose: This study aimed to investigate the feasibility of testing neonates using the SFI technique, establish normative SFI data for resonance frequency (RF) and mobility of the middle ear in terms of changes in sound pressure level (ΔSPL in dB), and describe the dynamics of the middle ear in healthy Australian neonates.

Study Sample: A prospective sample of 100 neonates (58 males, 42 females) with mean gestational age of 39.3 wk (SD = 1.3 wk, range = 38-42 wk), who passed all three tests, namely, automated auditory brainstem response, transient evoked otoacoustic emissions and 1000-Hz tympanometry, were included in this study.

Data Collection and Analysis: A SFI research prototype was used to collect the data. First, the sound pressure level (SPL) in the ear canal was measured as a probe tone frequency was swept from 100-2000 Hz with the ear canal static pressure held constant at 200 daPa. Then, this measurement was repeated with the static pressure reduced in 50 daPa steps to -200 daPa. Additional measurement was also performed at the static pressure where the peak of the 1000 Hz tympanogram occurred. A graph showing the variation of SPL against frequency at all static pressures was plotted. From this graph, the RF and ΔSPL at tympanometric peak pressure (TPP) were determined. Descriptive statistics and an analysis of variance (ANOVA) were applied to the RF and Δ SPL data with gender and ear as independent variables.

Results: The results showed two resonance regions of the outer /middle ear with the high RF (mean = 1236 Hz, 90% range: 830-1518 Hz) being approximately equal to four times that of the low RF (mean = 287 Hz, 90% range: 209-420 Hz). The low RF was more easily identifiable than the high RF. The ΔSPL at the low RF (mean = 8.2 dB, 90% range: 3.4-13

dB) was greater than that at the high RF (mean = 5.0 dB, 90% range: 1.5-8.1 dB). There were no significant differences or interactions between genders and ears.

Conclusion: The study showed that the SFI is a feasible test of middle ear function in neonates. The SFI results revealed two regions of resonance with the lower resonance (287 Hz) possibly related to the movements of the outer ear canal wall and higher resonance (1236 Hz) related to the resonance of the middle ear. The normative data developed in this study will be useful in evaluating outer and middle ear function in neonates.

Key words: Sweep frequency impedance, resonance frequency, volume displacement, high frequency tympanometry, multifrequency tympanometry, middle ear, neonate.

Abbreviations:

AABR = automated auditory brainstem response;
ANOVA = analysis of variance;
daPa = deca Pascal;
DPOAEs = distortion product otoacoustic emissions;
Δ = delta;
HFT = high-frequency tympanometry;
MFT = multifrequency tympanometry;
pkSPL = peak sound pressure level.
Ps = static pressure;
RF = resonance frequency;
SFI = sweep frequency impedance;
SPL = sound pressure level;
TEOAEs = transient evoked otoacoustic emissions;
TPP = tympanometric peak pressure;
WAI = wideband acoustic immittance;
WBR = wideband reflectance;

2.3 Introduction

Tympanometry is a standard diagnostic tool used in the assessment of middle ear function in children and adults. Conventional tympanometry measures the compliance of the middle ear system using a single low frequency probe tone, usually 226 Hz, while varying the air pressure in the ear canal (Jerger, 1970; Margolis and Shanks, 1984). However, conventional tympanometry is not recommended for infants younger than 7 mo because of its poor sensitivity in identifying middle ear pathologies in this population (Paradise et al, 1976; Rhodes et al, 1999; Purdy and Williams, 2002; Baldwin, 2006). Conventional tympanometry in infants younger than 7 mo has been shown to produce erroneous findings, such as normal tympanograms in ears with confirmed middle ear effusion (Paradise et al, 1976; Hunter and Margolis, 1992; Meyer et al, 1997; Purdy and Williams, 2002; Baldwin, 2006) and abnormal results on tympanograms indicating middle ear effusion in neonates with normal middle ear function (Keefe and Levi, 1996; Rhodes et al, 1999).

The poor sensitivity of conventional tympanometry in young infants compared to the adults has been attributed to the anatomic and acoustic differences between the neonate and adult outer and middle ear systems (Keefe and Levi, 1996; Hunter and Margolis, 2011). First, unlike the adult's stiffness-dominated middle ear system, the middle ear of a neonate is mass-dominated (Holte et al, 1991; Keefe and Levi, 1996; Meyer et al, 1997).

Second, there are differences in the anatomy of the outer and middle ear between a neonate and an adult (Himelfarb et al, 1979; Sprague et al, 1985; Hunter and Margolis, 2011). A neonate has an external ear canal that is smaller in length and diameter than that of an adult (Wilson, 2012), an altered orientation of the tympanic membrane with respect to the ear canal, and thickened tympanic membrane due to the presence of mesenchymal tissues (Ruah et al, 1991). As the osseous portion of the ear canal is not rigid in newborns, its movement can influence the shape of the tympanogram (Paradise et al, 1976). Distension of the ear canal wall to pressure pulses has also been noted for infants up to age 2m (Holte et al, 1990; Holte et al, 1991). Based on the responses to sound in the 220-660 Hz using wideband reflectance technology, Keefe et al (2003) indicated significant motions and resonant amplifications of the ear canal wall in infants.

Third, the mass dominance in the infant middle ear significantly increases the ear canal impedance and reflection responses, thereby reducing the energy transfer into the infant's middle ear compared to the adult (Keefe et al, 1993). The energy transmission into the middle ear of infants is most efficient in the 1000-4000 Hz range with the 220-660 Hz range being least efficient (Keefe et al, 1993; Keefe et al, 2003). Additionally, the resonance frequency (RF) of the mass-dominated middle ear system of infants has been reported to be lower than that in the adult middle ear system (Weatherby and Bennett, 1980; Holte et al, 1991; Kei et al, 2010; Kei and Zhao, 2012). Consequently, the mathematical principle underlying the tympanometric measurements of stiffness-dominated adult middle ear systems is not applicable to neonates (Margolis and Hunter, 2000). Thus, the standard low frequency probe tone is not suitable for the assessment of middle ear function in young infants (< 7 mo) (Keith, 1975; Paradise et al, 1976; Hunter and Margolis, 1992; Keefe et al, 1993; Meyer et al, 1997). Therefore, it is important to further investigate other tests that may be suitable for the assessment of the middle ear of infants.

In the last decade, alternate measures such as high frequency tympanometry (HFT) using a 1000 Hz probe tone (Purdy and Williams, 2002; Kei et al, 2003; Margolis et al, 2003; Swanepoel et al, 2007) and wideband acoustic immittance (WAI) measures (Keefe et al, 1993; Shahnaz, 2008; Keefe, 2008 ; Werner et al, 2010) have been advocated as tests of middle ear function in neonates and young infants. Although there are two commercial manufacturers of WAI equipment (Mimosa and Interacoustics) with FDA clearance in the USA, they are presently not used routinely in clinics. Normative HFT data are available in neonates (Kei et al, 2003; Margolis et al, 2003; Calandruccio et al, 2006; Mazlan et al, 2007), but there are still unresolved issues regarding their measurement and interpretation (Kei and Zhao, 2012). In addition, studies that have compared the test performance of HFT and wideband reflectance (WBR) with distortion product otoacoustic emissions (DPOAEs) have found that WBR predicted DPOAEs outcomes more accurately than HFT measures (Sanford et al, 2009; Hunter et al, 2010). Because of the limitations of currently available technologies used in the assessment of middle ear function in infants, it is important to explore alternate technologies.

The sweep frequency impedance (SFI), developed in the 1990s, measures the RF and volume displacement (Δ SPL) of the outer and middle ear system at tympanometric peak pressure (TPP, the pressure at which the admittance attains a maximum) (Wada and

Kobayashi, 1990; Zhao et al, 2002; Murakoshi et al, 2012). In a broader sense, although both SFI and WAI utilise a wider frequency range for measurements, they assess different aspects of middle ear function. WAI assesses wideband acoustic transfer functions of the middle ear (Feeney and Sanford, 2012). In part, WAI measures are attractive because they measure middle ear transfer function over a wide frequency range from 0.25-8 kHz for adults and up to 20 kHz for infants (Keefe et al, 1993). Additionally, it also measures the middle ear transfer function to include bandwidth of speech (Feeney and Sanford, 2012). On the other hand, SFI does not measure the admittance of the middle ear system. It measures the dynamic behaviour of the outer and middle ear system by investigating how the RF and Δ SPL change when the ear canal pressure deviates from the TPP (Wada et al, 1989; Murakoshi et al, 2012). When the ear canal pressure is greater than the TPP, the RF increases and Δ SPL decreases, indicating greater stiffness of the outer and middle ear of the healthy infant. When the ear canal pressure is smaller than the TPP, the RF increases and Δ SPL decreases at a much faster rate until the ear canal collapses under significant negative ear canal pressure.

Sound energy is transmitted most efficiently at the middle ear RF because the ear drum vibrates with the largest displacement amplitude at that frequency. Using finite-element method, Koike et al (2000) showed that the maximum value of the middle ear transmission gain (i.e. forward and backward transmission) is obtained at the RF. The peak value of the gain depends on the mobility of the middle ear (i.e. the damping component of the middle ear impedance). Such middle ear dynamic characteristics can be easily measured in terms of RF and the mobility of the middle ear (Δ SPL) using a SFI metre (Zhao et al, 2003). Wada et al (1995) and Zhao et al (2003) suggested that the dynamic characteristics of the outer and middle ear can provide further insight into pathological conditions in patients with conductive disorders.

Whereas the SFI has been reported to be useful in the diagnosis of various middle ear pathologies in children and adults, its application to evaluating the middle ear function in neonates is only just emerging (Wada et al, 1989; Wada and Kobayashi, 1990; Wada et al, 1998; Zhao et al, 2002; Murakoshi et al, 2012).

Nonetheless, the knowledge of the dynamic characteristics of the middle ear in neonates is limited. The paucity of SFI studies on neonates can be attributed to the large size of the probe in the original research prototype developed by Wada et al (1989) that could not

be used with neonates. In the present study, a modified version of research SFI unit developed by Murakoshi et al (2013) was used. This new SFI unit uses a small probe suitable for neonates. The validity of SFI has been confirmed with examination of frequency characteristics of probe using calibration cavities and comparison of SFI test in normal hearing adults and healthy neonates as detailed by Murakoshi et al (2013). The present study used the same unit developed and calibrated by Murakoshi et al (2013). Apart from a few case reports that have utilised the new unit (Kei et al, 2010; Kei and Zhao, 2012), to date, no studies have yet systematically investigated SFI in neonates. These case reports show that SFI can be used as a clinical tool to determine if the dynamic characteristics of the middle ear are normal or not. For example, in healthy neonates, Murakoshi et al. (2013) noted two RF regions, at 260 (low RF) and 1130 Hz (high RF), respectively. In the other study, Murakoshi et al (2012) noted that the high RF disappeared in neonates with middle ear dysfunction.

Given the above useful clinical information, SFI has the potential to detect middle ear disorders in neonates. However, before it can be used as a diagnostic assessment tool for middle ear function, investigation into the feasibility and utility of the SFI in neonates must occur. The aims of the present study were to determine the feasibility of using new SFI unit developed by Murakoshi et al (2013) for evaluation of outer and middle ear dynamics in neonates and establish normative SFI data for RF and Δ SPL in healthy Australian neonates.

2.4 Methods

2.4.1 Participants

Recruitment of participants was performed by nurses of the Healthy Hearing team in accordance with the ethical guidelines approved by the Human Research Ethical Committee of Townsville Health Service District and the University of Queensland Behavioural and Social Science Ethical Review Committee (Appendix 1). Parents of healthy neonates at the maternity unit of The Townsville Hospital were informed of the study prior to hearing screening (Appendix 2). Participants were included in the study upon written consent from their parents. Due to the working roster of dedicated screeners in the project, consenting and data collection were limited to specific times of the day. Therefore, all healthy neonates born at the hospital were not available for recruitment.

A total of 100 neonates (58 males, 42 females), were included in this study. Only ears that passed all the three tests in a test battery, namely, automated auditory brainstem response (AABR), transient evoked otoacoustic emissions (TEOAEs) and HFT, were included in this study. Only one ear per neonate was included in the analysis. In cases where both ears of a neonate passed the test battery, either the right or left ear was selected using a random table. All participants were born full-term with a mean gestational age of 39.30 wk (SD = 1.3 wk, range = 38-42 wk), with an uneventful birth history and no high risk factors for hearing loss (Joint Committee on Infant Hearing, 2007). The mean age at the time of testing was 43.9 hr (SD = 18.5, range = 8-103 hr). The mean birth weight was 3522 g (SD = 433.3, range 2290-4870 g).

2.4.2 Procedure

Testing was done in a quiet room of the maternity ward of The Townsville Hospital, Queensland, Australia, where the ambient noise level was less than 40 dB A. Trained nursing staff performed the hearing screening using AABR while a clinical audiologist administered HFT, TEOAEs, and SFI tests to the neonates who passed AABR screening.

The neonates were tested while in a state of natural sleep or while awake, but quiet and settled. The entire test battery took an average of 30 minutes for both ears for a well settled neonate. Wherever possible, the HFT and TEOAEs tests were completed for both ears of each infant with no particular test order. The most accessible ear was tested first.

2.4.3 Test battery

AABR screening was always performed first as part of universal newborn hearing screening. This was necessary to ensure likelihood of functionally normal hearing. The AABR screening was done using the ALGO3 newborn hearing screener (Natus Medical Inc.) with clicks presented at a level of 35 dB nHL. Results were visually displayed on the screen as either a pass or refer. A pass on the AABR indicates grossly normal auditory function up to the brainstem. All neonates included in the study passed the AABR screen in both ears.

HFT was performed with use of a Madsen Otoflex 100 acoustic immittance device (GN Otometrics) with a 1000 Hz probe tone. The admittance (Y) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec. The pass criteria were a single positively peaked tympanogram with the middle ear pressure between 50 and

-150 daPa and peak compensated static admittance (+200 daPa tail to peak) of at least 0.2 mmho (Mazlan et al, 2009).

TEOAEs were performed using a Scout sport system (Biologic Navigator Plus). Emissions were measured at 1000, 1500, 2000, 3000 and 4000 Hz. The signal consisted of wideband clicks of 80 μs duration, at a target amplitude of 80 dB pkSPL. The pass criteria included a reproducibility of 70% and difference of at least 3 dB between the amplitude of the emissions and associated noise floor in the one-third octave bands from 2000-4000 Hz (Kei et al, 2003; Vander Werff et al, 2007).

SFI was performed using a new SFI unit for testing neonates and infants developed by Murakoshi et al (2013). Figure 2.1a shows the block diagram of the new unit. This new SFI device consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, an air pump, a pressure sensor, and a pressure relief valve. The new probe used for testing neonates is smaller than the original one (Figure 2.1b). The diameter of the new probe is approximately 3 mm when compared to the original 5-mm conventional probe. There are three tubes in the 3-mm new probe. The first tube is for applying static pressure (Ps) to the ear canal. The second tube delivered sound to the external ear canal via an earphone. The third tube measured sound pressure in the external ear canal using a microphone. A specially designed cuff suitable for testing neonates was attached to the tip of the probe to obtain a hermetic seal during testing. This new SFI unit was controlled using LabView under WINDOWS. Figure 2.1c shows the photo of new SFI metre setup.

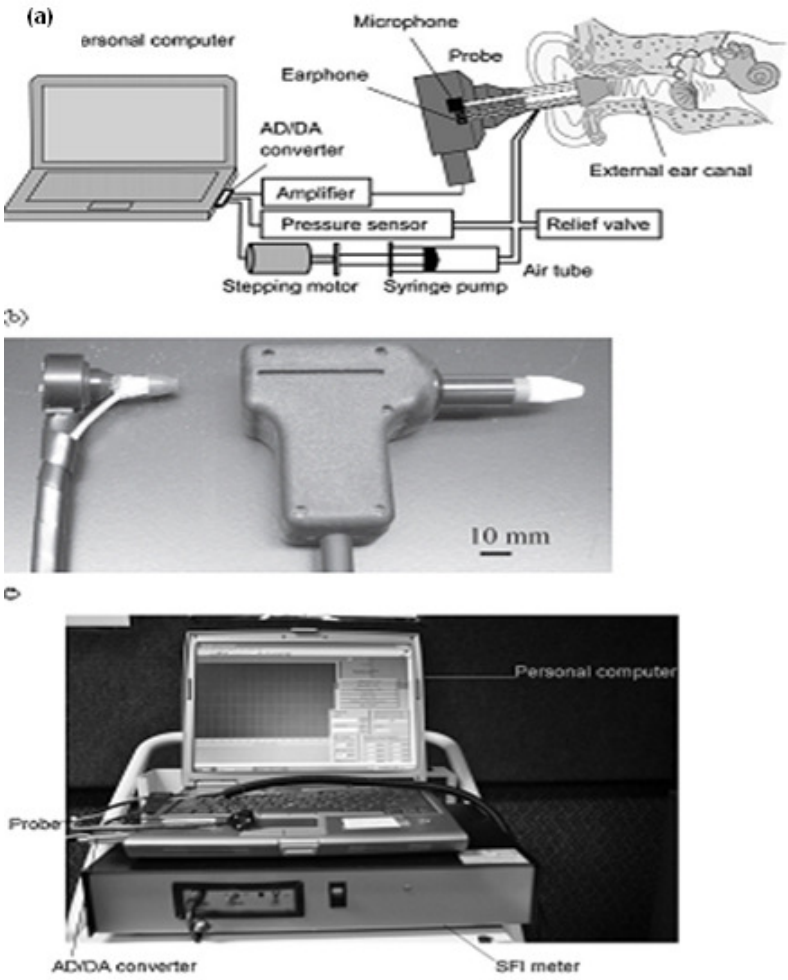

Figure 2.1. New SFI metre for testing neonates and infants. (a). Block diagram of the SFI metre. The SFI metre consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, a syringe pump, a pressure sensor and a relief valve. This new modified research prototype is controlled using LabView under WINDOWS. [From Murakoshi et al (2013).Int.J.Pediatr.Otorhinolaryngol. Copyright © 2012 by Elsevier Ireland Ltd. Reprinted with permission of Elsevier Ireland Ltd.]. (b) The SFI probes. Left – new probe for testing neonates and infants; Right – conventional probe for testing children and adults. (From Murakoshi et al (2012). Assessing middle ear function in infants (edited by J. Kei and F. Zhao), p124. (c) Photo of new SFI metre setup.

The calibration procedure of the SFI device has been described by Murakoshi et al (2013). A brief description is provided here. The frequency characteristic of the probe and its relationships between the input sound frequency and the SPL measured by the microphone (SPL curve) was obtained using a calibration cavity. The calibration cavity was made up of an air-filled plastic circular cylinder rigidly terminated at one end. The lengths of calibration

cavities were 5, 15, 25 and 35 mm, each with a diameter of 4 mm. To check the frequency characteristics of the new SFI probe, the calibration cavity was measured and the relationship between the SPL and frequency was determined. Figure 2.2 illustrates the measurement data from calibration. When the length of the calibration cavity was 5 mm, the measurement data were smaller than the numerical result. When the lengths of calibration cavity were 15, 25 and 35 mm, the measurement data were coincident with the numerical results, indicating the SPL decreased gradually with an increase in frequency. The slope of the SPL curve was large when the length of the calibration cavity was large.

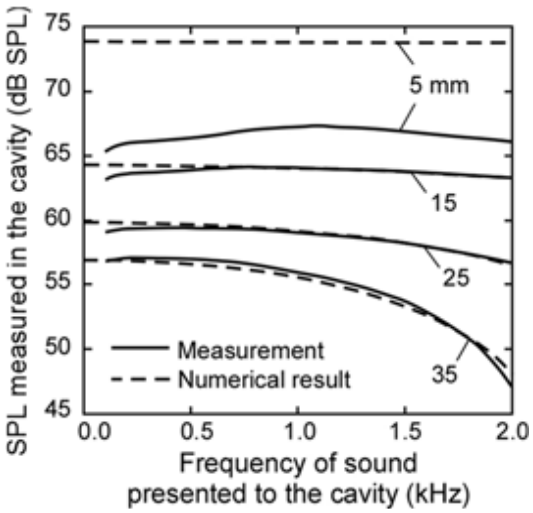

Figure 2.2. SFI results of calibration cavities for new modified research prototype. The lengths of the calibration cavity lc were 5, 15, 25 and 35 mm and their diameters were 4 mm. Except for the data obtained from the short cavity with a length of 5 mm, the SFI results corresponded to the numerical results. i.e., the sound pressure decreased gradually with an increase in the frequency and the ratio of sound pressure decrease was large when the length of an air-filled circular cylinder was large. [From Murakoshi et al (2013). Int. J. Pediatr. Otorhinolaryngol. Copyright 2012 by Elsevier Ireland Ltd. Reprinted with permission of Elsevier Ireland Ltd.]

The SFI test was performed using following steps. A probe tip was inserted into the ear canal using a modified cuff and a tight seal was obtained. First, a HFT was performed using 1000 Hz probe tone and the static compliance and the TPP were recorded. Second, the sound pressure level in the ear canal was measured as the probe tone frequency was swept from 100-2000 Hz while the external auditory canal static pressure (Ps) was held constant at

200 daPa. Third, this measurement was repeated with Ps reduced in 50 daPa steps down to -200 daPa. Additional measurements were also performed at the TPP. The entire SFI procedure was automated, and once the seal was obtained, it took about one minutes to complete the test in each ear. The sweeping probe tone level was kept below 75 dB SPL, which is below the stapedial reflex threshold. If the neonate woke up or was unsettled due to the application of ± 200 daPa static pressure, testing was stopped and repeated when the infant became settled. In addition to this, the SFI unit has inbuilt mechanical pressure relief value as well as provision to measure loss of pressure before and after the sweep.

2.5 Results

A total of 195 neonates were initially enrolled in this study. Out of the 195 neonates, 95 were not included in the study either because the data were incomplete, or the infants did not pass all of the three tests. Although difficulty in obtaining a tight probe seal contributed to some incomplete test data, the main reason for not completing the tests was lack of time. The hospital's policy of discharging normal babies within 24 hr of delivery that led to nearly 50% of subjects being lost to the study after enrolment. Of the 100 neonates included in the study, 25 passed the test battery in the left ear, 33 in the right ear and the remaining 42 in both ears. For the 42 neonates who passed the test battery in both ears, data from either right or left ear was chosen with the use of a random table so that only one ear per neonate was considered for the study. Overall, the study included 100 ears from 100 neonates (57 right and 43 left ears).

The dynamic behaviour of the outer and middle ear refers to the change in RF and Δ SPL as the ear canal pressure is changed from positive to negative pressure. In the present study, SFI result is measured at TPP. The SFI result is represented by the Δ SPL variation as function of frequency at various Ps. The Δ SPL reflects the volume displacement and is an indicator of the mobility of the middle ear system in terms of changes in sound pressure in the RF region (Wada and Kobayashi, 1990). Figure 2.3 shows the typical SFI results obtained from a healthy one-day-old male neonate who passed the test battery in the left ear.

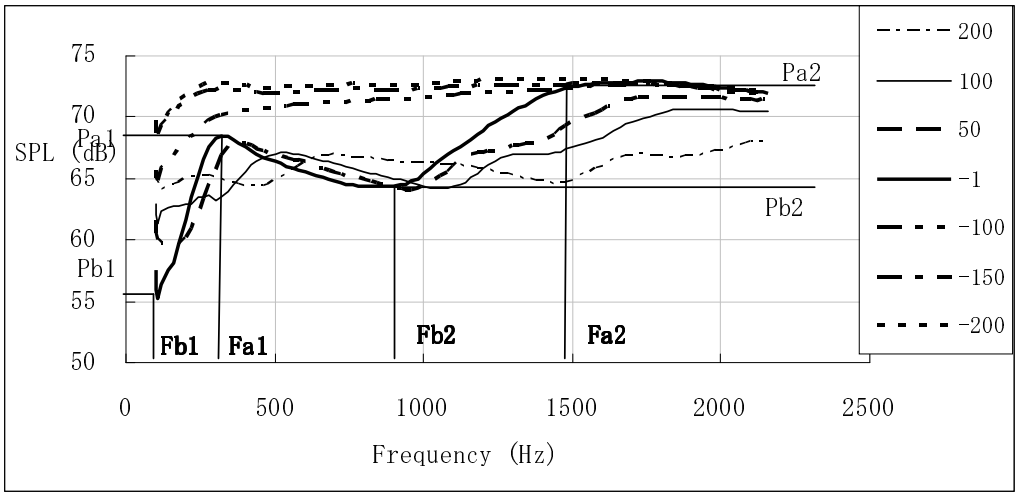

Figure 2.3. Example of SFI results obtained from the left ear of a one-day-old male neonate who passed the test battery. The SPL curve at -1 daPa (Ps) shows two variations in sound pressure. The maximum and minimum SPL (i.e., Pa1 and Pb1; Pa2 and Pb2, respectively) as well as frequencies corresponding to these sound pressures (i.e., Fa1 and Fb1; Fa2 and Fb2, respectively) are chosen from visual inspection of the SPL curve.

As demonstrated in Figure 2.3, the RF and Δ SPL are measured directly from the SPL curve at static middle ear pressure of -1 daPa (TPP). At the frequency where sound pressure curve (-1 daPa curve) varies considerably, the tympanic membrane volume displacement (Δ SPL) is largest, and this is considered to be the RF of the outer/middle ear (Murakoshi et al, 2012). Previous studies (Wada et al, 1993; 1995) have shown that the largest volume displacement (Δ SPL) of the ear drum is at about the median frequency between the frequencies (F_a1 and F_b1; F_a2 and F_b2, respectively in Figure 2.3) corresponding to the maximal and minimal sound pressures (P_a1 and P_b1; P_a2 and P_b2, respectively in Figure 2.3). According to Wada et al (1993), the RF is not exactly equal to the median frequency because of damping effects. However for simplicity, the median frequency is considered as the resonance frequency of the outer/middle ear (Wada et al, 1993; Zhao et al, 2003). It has also been confirmed that the difference between P_a1 and P_b1, and P_a2 and P_b2 indicates the volume displacement of tympanic membrane at resonance (Wada et al, 1993; Murakoshi et al, 2013). The sound pressure changes can be observed at two frequency regions in the SPL curve. The maximal and minimal SPL (i.e., P_a1 and P_b1; P_a2 and P_b2, respectively), as well as frequencies corresponding to these sound pressures (i.e., F_a1 and F_b1; F_a2 and F_b2, respectively), were chosen from visual inspection of the SPL curve. Although they could also be measured using an automated mathematical procedure after converting all data into a

digital format, this procedure was not used. Once these points are selected, both RF and Δ SPL are calculated using following equations (Wada et al, 1989; Murakoshi, 2012).

Low Resonance Frequency, RF1 = $(F_a1+F_b1) / 2$ (2.1)

High Resonance Frequency, RF2 = $(F_a2+F_b2) / 2$ (2.2)

SPL change at RF1, Δ SPL1 = $P_a1 - P_b1$ (2.3)

SPL change at RF2, Δ SPL2 = $P_a2 - P_b2$ (2.4)

As seen in Figure 2.3, when Ps = - 1 daPa, the Δ SPL curve (shown in thick line) shows variations in SPL at two frequency regions: between 150 and 300 Hz, and between 800 and 1500 Hz, respectively. These two frequency regions are considered as the RF regions of the outer/middle ear (Murakoshi et al, 2013). The low resonance (RF1) in this infant occurred at 250 Hz with Δ SPL1 of 12 dB and the higher resonance (RF2) occurred at 1200 Hz with Δ SPL2 of 8 dB.

By comparing SFI results with multifrequency tympanometry (MFT) findings as probe tone frequency increased from low frequency to high frequency in a normal hearing subject, Wada et al (1998) demonstrated that when Ps = 0 daPa (ambient middle ear pressure), maximal variation in sound pressure level (SPL) occurred at RF. For instance, in Figure 2.3, volume displacement is largest at two instances where the pressure curve (Ps = -1 daPa curve) varies considerably (P_a1-P_b1 and P_a2-P_b2, respectively). The frequencies corresponding to these changes in sound pressures (F_a1 and F_b1; F_a2 and F_b2, respectively) are considered to be the resonant frequencies of outer (RF1) and middle ear (RF2). RF1 and RF2 are determined as shown in equations (1) and (2).

When the pressure difference between the bottom of the curve and its peak (denoted Δ SPL) is large, the volume displacement at RF is large and vice versa (Ps = -1 daPa curve in figure 2.3). Δ SPL indicates the degree of mobility of the tympanic membrane. Maximal mobility is recorded at the middle point of this curve at two places in Figure 2.3 (Δ SPL1 and Δ SPL2). In this study, the mid-point of the frequencies at the peak and bottom of the SPL curve is considered as the RF (RF1 and RF2) of outer and middle ear respectively (Wada et al, 1995; Wada et al, 1993; Zhao et al, 2003; Murakoshi et al, 2013).

At TPP (-1 daPa), the SPL curve shows a typical pattern of two variations of the sound pressure in a healthy neonate (see the thick solid line in Figure 2.3). As the static air pressure was increased to 50 daPa, both RF1 and RF2 increased (i.e., the two variations of sound pressure were shifted to the right) while both Δ SPL1 and Δ SPL2 decreased, indicating an increase in stiffness of the outer/middle ear. Further increase of the static pressure to 100 and 200 daPa led to further increase in RF1 and RF2, indicating further increase in stiffness. This dynamic behaviour of the outer/middle ear is typical of the healthy neonate ear.

When a negative static pressure of -100 daPa was applied to the external auditory canal, the SPL curve did not show much variation (Figure 2.3). Instead, a flat response with an overall increase in sound pressure to 70 dB SPL was observed. Further decrease of static pressure to -150 and -200 daPa led to similar flat responses with an increased overall sound pressure to 72.5 dB SPL, similar to one observed in the calibration cavity.

Table 2.1 shows the mean, SD, and the 5th, 50th, and 95th percentiles for RF1, RF2, Δ SPL1 and Δ SPL2, respectively, for 100 healthy neonates who participated in this study. Overall mean RF2 (1236.1 Hz) was approximately equal to four times that of RF1 (287.1 Hz). The RF1 was more easily identified and measured than the RF2 because of the greater variation of the SPL at RF1 than at RF2 (Figure 2.3). However, when inflection was small, the precise determination of the position of F_a2, F_b2, P_a2 and P_b2 was difficult because of spread of RF2. This happened in 11 infants. Although the SD for RF1 (68.5 Hz) appeared to be smaller than that for RF2 (200 Hz), the coefficient of variation (defined as SD/mean) for RF1 (0.24) was greater than that for RF2 (0.16), indicating that the dispersion of values about the mean resonance frequency is actually greater for RF1 than for RF2. The overall mean Δ SPL1 (8.2 dB) at RF1 was greater than that at RF2 (5.0 dB). The coefficient of variation for Δ SPL1 (0.38) was smaller than that for Δ SPL2 (0.40), indicating that the dispersion of values about the mean Δ SPL is slightly smaller for Δ SPL1 than for Δ SPL2.

Table 2.1. Normative data for 100 neonates (58 males, 42 females) who passed AABR, HFT and TEOAE tests

Variable	Ear	N	Mean	SD	5%le	50%le	95%le
RF1 (Low resonance frequency) in Hz	R	57	290.2	66.7			
	L	43	283.1	71.3			
	Total	100	287.1	68.5	208.5	260.0	419.5
SPL1 (Change in SPL at RF1) in dB	R	57	8.2	3.0			
	L	43	8.3	3.2			
	Total	100	8.2	3.1	3.4	8.0	13.0
RF2 (High resonance frequency) in Hz	R	57	1226.3	208.4			
	L	43	1249.1	189.8			
	Total	100	1236.1	200.0	829.5	1239.5	1518.1
SPL2 (Change in SPL at RF2) in dB	R	57	5.2	2.1			
	L	43	4.7	2.0			
	Total	100	5.0	2.0	1.5	5.0	8.1

An analysis of variance (ANOVA) was applied to the RF1 data, with gender and ear as independent variables. The results showed no significant main effects for gender and ear nor Gender × Ear interaction ($p > 0.05$). The ANOVA analysis was repeated separately for the dependent variables RF2, Δ SPL1 and Δ SPL2. The results showed no significant gender or ear effects and no significant interactions for these variables ($p > 0.05$). Hence, the data were pooled across genders and ears.

The 90% range (95th percentile – 5th percentile) of the RF1, RF2, Δ SPL1 and Δ SPL2 (Table 2.1) is recommended as the normal range for the dynamic characteristic properties of the outer/ middle ear in healthy neonates respectively. Any deviation from this 90% range may indicate possible disturbance in outer/middle ear dynamics. Further studies using test performance of SFI in ears with and without middle ear dysfunction are needed to determine whether this normative range is best suited for neonates.

The above results were compared with the findings of Murakoshi et al (2013) who obtained SFI data from 9 neonates (10 ears) in Japan using new SFI metre. Table 2.2 shows no significant difference between the two studies for all measures except Δ SPL2. Statistical analysis showed a significantly higher Δ SPL2 value than that of the Murakoshi et al (2013) study (5.0 versus 3.6 dB) which could be due to sampling error as Murakoshi et al (2013) study had very small sample size (10 ears).

Table 2.2. Comparison of SFI findings between the Murakoshi et al (2013) (N = 10 ears) and present study (N = 100 ears) using a two sample t-test.

Measure	Mean	SD	t	df	p
RF1 (present study)	287.1	68.5	1.235	108.0	0.220
RF1 (Murakoshi et al, 2013)	260.0	30.0			
RF2 (present study)	1236.1	200.0	1.644	108.0	0.103
RF2 (Murakoshi et al, 2013)	1130.0	120.0			
ΔSPL1 (present study)	8.2	3.1	-1.043	108.0	0.299
ΔSPL1 (Murakoshi et al, 2013)	9.3	2.2			
ΔSPL2 (present study)	5.0	2.1	2.154	108.0	*0.0335
Δ SPL2 (Murakoshi et al, 2013)	3.6	1.9			

*$p<0.05$

2.6 Discussion

The present study provided normative SFI measures in healthy Australian neonates who passed a test battery (Table 2.1). The RF and Δ SPL were obtained using a new SFI system specially designed for neonates and infants.

This study used a combination of tests to determine middle ear status in neonates. Past studies have used either TEOAEs or DPOAEs as the reference standard to determine normal middle ear function in neonates (Kei et al, 2003; Margolis et al, 2003; Calandruccio et al, 2006; Mazlan et al, 2007; Sanford et al, 2009). TEOAEs and DPOAEs, however, are not a perfect gold standard. Studies have shown that use of OAEs has limitations as a test of middle ear function as they have been found to be present in some ears with middle ear dysfunction in infants and children (Driscoll et al, 2000; Kei et al, 2003; Kei and Zhao, 2012). Instead, a pass with a combination of tests may provide a more stringent reference standard for normal middle ear function (Kei and Zhao, 2012; Mazlan and Kei, 2012). Therefore, this study considered a pass in all the tests in a test battery to be the reference standards for normal middle ear function.

The results of present study showed two regions of resonance, one at low frequency (RF1) and another at high frequency (RF2) (Figure 2.3). Although this finding is in contrast with other SFI studies on adults that found only one resonance region at about 1100 Hz (Wada and Kobayashi, 1990; Wada et al, 1998), it is consistent with more recent reports (Kei et al, 2010; Kei and Zhao, 2012; Murakoshi et al, 2012). In addition, the Murakoshi et al (2013) study provided clear evidence of the existence of two resonance regions using a model of a neonate's outer and middle ear system. The presence of two resonance frequency regions suggest that there are two vibrating elements in the neonatal outer and middle ear, thus, indicating the possibility of separate contributions from the outer and middle ears.

According to Murakoshi et al (2013), the resonance which occurs in the low frequency region (e.g., 250-300 Hz) may be associated with the movement of the elastic external ear canal wall. This proposition was confirmed by using a gel model mimicking the neonate's external ear canal. They showed a similar variation in SPL around 500 Hz. Unsurprisingly, this phenomenon occurs due the soft ear canal walls resonating in the presence of sound of a particular frequency (RF1). The elasticity of the ear canal wall can produce up to 70% change in ear canal diameter in response to pneumatic stimulation during the first five days of life (Holte et al, 1990; Holte et al, 1991). Similarly, based on model simulations on temporal bone data from a 22-day-old neonate, Qi et al (2006) reported 27%-75% change in ear canal volume over a pressure range of + 300 to – 300 daPa. In addition, Keefe et al (1993) noted that at frequencies below 500 Hz, less power is transferred into the middle ear of infants below 4 months due to ear canal wall vibrations and resonance.

The presence of a resonance in the low frequency region in young infants has also been reported in earlier studies using MFT (Holte et al, 1991; Meyer et al, 1997). For instance, Meyer et al (1997) measured the RF frequency of the ear of one infant from the age of two weeks to six and half months of age in a longitudinal study. They found that the RF remained below 550 Hz until the infant was 99 days old. Similarly, Holte et al (1991) reported low frequency resonance at approximately 450 Hz and another resonance at about 710 Hz in 16 of 43 ears of neonates. Holte et al also noted that, in the older groups, the lower resonance dropped out and higher resonance increased to more than 900 Hz. These findings of Meyer et al (1997) and Holte et al (1991) are consistent with the results of the present study where the low resonance frequency (RF1) with large Δ SPL1 can be easily identified in

neonates, whereas the high resonance frequency (RF2) with small Δ SPL2 is not as conspicuous as the low resonance frequency (see Figure 2.3).

The present study also identified a resonance region between 1100 and 1300 Hz in healthy neonates, consistent with the findings of Murakoshi et al (2012) and Murakoshi et al (2013). As the RF (RF2) of neonates is similar to the middle ear resonance frequency of adult ears, Murakoshi et al (2012) and Murakoshi et al (2013) ascribed the RF2 in neonates to the dynamic behaviour of the middle ear. This finding is in line with previous studies that have reported similar middle ear RF in adults using SFI equipment (Wada and Kobayashi, 1990). For example, Wada et al (1998) measured the RF in 275 adult ears with intact ear drums and normal hearing using the SFI device. They reported a mean RF of 1170 Hz (SD = 270 Hz) in adult ears, which is slightly lower than that reported in this study.

The results of the present study obtained from healthy neonates showed a mean low RF (RF1) of 287 Hz (SD = 68.5 Hz; 90% range = 209-420 Hz) and high RF (RF2) of 1236 Hz (SD = 200 Hz; 90% range = 830-1518 Hz). These RF normative data are consistent with that of the Murakoshi et al (2013) study, with no significant difference in RF between the two studies. The normative RF data established in the present study may be used as a guide for determining the conductive properties of the outer and middle ear.

Measurements of middle ear dynamic characteristics around the RF have been carried out previously (Colletti, 1976; Valvik et al, 1994). Colletti (1976, 1977) noted consistent changes in the shapes of tympanogram (impedance/pressure function) with different probe tone frequencies from 200-2000 Hz in 290 clients. In 80 clients with normal middle ear status, the shape of tympanogram changed in an orderly fashion as probe tone frequency increased. A V-shaped tympanogram was noted at the lowest frequencies, and a W-shaped tympanogram was observed from about 650-1400 Hz, which indicated the RF region of the middle ear. At higher frequencies, an inverted V shape was observed. Wada et al (1998) compared the change in shape of the tympanogram in MFT format as the probe-tone increased with that of sound pressure curves (Δ SPL) in SFI procedure in a normal hearing adult. They noted that the shape of tympanograms changed in an orderly fashion when the probe-tone frequency increased. They recorded a V-shaped tympanogram in the frequency range below the RF, a W-shaped tympanogram in the range that contained the RF and an inverted V shape in the range that was above the RF. The RF of the middle ear ranged from

630-1710 Hz (95% confidence interval) in their study with a mean value of 1170 Hz (SD = 270 Hz). Although SFI uses a different technique from MFT tympanometry, the SFI results correlated with MFT data.

Apart from the RF, the present study provided normative data for Δ SPL, which is considered as an index of middle ear mobility in the RF region. In the present study, the Δ SPL was larger at RF1 (mean Δ SPL1 = 8.2 dB) than at RF2 (mean Δ SPL2 = 5.0 dB). When compared to the findings of Murakoshi et al (2013), no difference was seen in Δ SPL1 between the two studies. However, the mean Δ SPL2 of the present study was significantly greater than that of the Murakoshi et al (2013) study. This discrepancy in Δ SPL2 may be accounted for by differences in race of the study samples and sample size between the two studies. In addition, errors in measuring Δ SPL2 may also contribute to such discrepancy when the inflection was small (Figure 2.3). However, these measurement errors can be reduced through the use of an automated procedure after converting all data to a digital format rather than reliance on visual inspection.

Since the SFI provides information about the dynamics of the outer and middle ear in neonates, it can assist in the interpretation of other middle ear measures, especially tympanometry. Because the low frequency resonance in neonates, as seen in the present study, can be attributed to the motion of the ear canal wall, it is safe to assume that 226 Hz tympanometry is not reliable in neonates as it is close to RF1. According to Kei et al (2003), about half of the neonates produced double peaked tympanograms in conventional tympanometry which made interpretation difficult. This supports the notion that conventional tympanometry is not suitable for testing neonates.

The present study provided valuable information about the dynamic behaviour of the outer and middle ear system in neonates. At ambient pressure, the SPL curve of a 1-day-old neonate shows a typical pattern of two variations of the sound pressure of a healthy neonate (see the thick solid line in Figure 2.3). As the static air pressure was increased to 50 daPa, both RF1 and RF2 increased (i.e., the two variations of sound pressure were shifted to the right) while both Δ SPL1 and Δ SPL2 decreased. Further increase of the static pressure to 100 and 200 daPa led to further increase in RF1 and RF2, suggesting that the stiffness of the neonate's outer and middle ear system increased with increasing positive static pressure

(Murakoshi et al, 2013; Murakoshi et al, 2012). Moreover, the SFI results indicate that the ear canal wall of a neonate is not rigid even at 200 daPa.

When a negative static pressure of -100 daPa was applied to the external auditory canal, the SPL curve did not show much variation (Figure 2.3). Instead, a flat response with an overall increase in sound pressure to 70 dB SPL was observed. Further decrease of static pressure to -150 and -200 daPa led to similar flat responses with an increased overall sound pressure to 72.5 dB SPL. Although not diagnostically confirmed, these results suggest that the ear canal probably collapsed at around 5 mm from the probe tip as reported by Murakoshi et al (2013), resulting in a similar response obtained in the 5 mm calibration cavity (Figure 2.2). These results also suggest that the external ear canal might have collapsed under a negative static pressure of -100 daPa. Similar findings of ear canal collapse have been reported by Holte et al (1991), Keefe et al (1993), Qi et al (2006), Sanford et al (2009) and Murakoshi et al (2013). This dynamic behaviour of a neonate's ear under negative static pressures indicates that there is no need to reduce the static pressure beyond -100 daPa when assessing middle ear function in normal neonates.

The present study did not find any gender or ear effects in all SFI measures in healthy neonates. Previous SFI studies in adults have not studied any gender effects (Wada et al, 1989; Wada et al, 1992; Wada et al, 1993; Wada et al, 1994; Wada et al, 1995; Wada et al, 1998; Zhao et al, 2002), although significant gender and ear effects were observed in some measures of middle ear function using HFT and wideband reflectance techniques (Keefe et al., 2000; Kei et al., 2003).

The measurement of SFI was relatively easy in well settled or asleep neonates. SFI provides additional information about the outer and middle ear dynamics in neonates and this information can be useful in the differentiation of outer and middle ear status in neonates. SFI techniques also allow us to measure the collapse of ear canal in infants under negative external auditory canal pressure. Therefore, SFI appears to be a feasible test of outer and middle ear dynamics in neonates. Further research is needed on SFI in ears with and without outer and middle ear dysfunction to determine its diagnostic efficacy.

2.6.1 Limitations

One of the limitations of the present study is the lack of a "true" gold standard for confirmation of middle ear status in neonates. While myringotomy is not ethical in neonates, pneumatic otoscopy is not accurate for this population (Jaffe et al, 1970). The presence of TEOAEs does not guarantee normal middle ear function as infants and children with subtle middle ear function can pass this test (Driscoll et al, 2001; Kei et al, 2003). Similarly, a pass in AABR does not rule out mild middle ear dysfunction in infants (Aithal et al, 2012). Therefore, a test battery approach was used in the present study to evaluate middle ear status.

Although it was relatively easy to test well settled neonates, SFI measurements took approximately 1-2 min to test one ear. Further improvement in instrumentation to reduce the test time is necessary if the SFI test is to be used as an assessment tool in audiology clinics, especially in newborn screening and diagnosis.

One possible source of error in SFI measures is the difficulty in measuring RF2 and Δ SPL2, when the inflection was small. In few infants the inflection was small; therefore, precise determination of the position of F_a2, F_b2, P_a2 and P_b2 was difficult. However, this difficulty can be overcome by having an audiologist with experience in SFI to perform the test and interpret the results. The measurement error can also be reduced significantly by calculating the position of F_a2, F_b2, P_a2 and P_b2 using an automated mathematical procedure after converting all data to a digital format.

Another potential disadvantage of the SFI is the need for repeated sweeps as the pressure is adjusted in steps. SFI techniques requires multiple pressurizations, which may not be desirable in the neonate and infant ears, as pressure changes can be a source of discomfort and require maintaining a probe seal for repeated sweeps. Since each sweep is essentially an independent measure, the reliability of the relative measures (comparing SPL curves across different sweeps) may be difficult when probe seal is not maintained and may introduce variable artifact. However, this can be overcome by measuring the loss of pressure before and after the sweep.

Lastly, inadequate probe seal and general movements of neonates during the SFI test may introduce artifact in the SPL tracings, making the interpretation of data more difficult. In a few neonates who were awake and wiggly, the test had to be aborted and restarted. Although the probe tips used in the present study were specially designed for neonatal ears,

they did not fit the ear canals snugly for all neonates. To this end, improvements in the probe tip design may be required.

2.6.2 Summary and directions for future research

In summary, the characteristics of SFI findings for neonates with normal TEOAEs, HFT and AABR results have been described in the present study. The SFI results showed two distinct resonance regions indicating functional differences in the outer and middle ear separately. The present study also established normative SFI data for low resonance frequency (RF1), high resonance frequency (RF2), and changes in SPL (Δ SPL1 and ΔSPL2) at the resonance frequency in healthy neonates with normal middle ear function.

Further research should investigate the dynamic behaviour of the outer and middle ear in preterm and full-term infants in the first 6 mo of age. Using a longitudinal experimental design, the study will provide insight into the developmental changes in dynamic properties of the outer and middle ear of young infants. Investigation into the test performance (sensitivity and specificity) of the SFI test in comparison to the other middle ear assessment methods is also desirable. Additional research is needed to determine the data set for disordered ears of young infants and address the issues of improving the test performance of the SFI instrument.

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2.8 References

Aithal S, Aithal V, Kei J, Driscoll C. (2012) Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia. *J Am Acad Audiol* 23(9):673-685.

Baldwin M. (2006) Choice of probe tone and classification of trace patterns in tympanometry undertaken in early infancy. *Int J Audiol* 45(7):417-427.

Calandruccio L, Fitzgerald TS, Prieve BA. (2006) Normative multifreqeuncy tympanometry in infants and toddlers. *J Am Acad Audiol* 17(7):470-480.

Colletti V. (1976) Tympanometry from 200 to 2000 Hz probe tone. *Audiology* 15(2):106-119.

Colletti V. (1977) Multifrequency tympanometry. *Audiology* 16(4):278-287.

Driscoll C, Kei J, McPherson B. (2000) Transient evoked otoacoustic emissins in six year old school children: A normative study. *Scand Audiol* 29(2):103-110.

Driscoll C, Kei J, McPherson B. (2001) Outcomes of transient evoked otoacoustic emission testing in six year old school children: A comparision with pure tone screening and tympanometry. *Int J Pediatr Otorhinolaryngol* 57(1):67-76.

Feeny MP, Sanford CA. (2012) Application of wideband acoustic transfer functions to the assessment of the infant ear. In: Kei J, Zhao F, (eds). *Assessing Middle Ear Function in Infants.* San Diego: Plural, 131-161.

Himelfarb MZ, Popelka GR, Shanon E. (1979) Tympanometry in normal neonates. *J Speech Lang Hear Res* 22:(1)179-191.

Holte L, Cavanaugh RM, Margolis RH. (1990) Ear canal wall mobility and tympanometric shape in young infants. *J Pediatr* 117(1 Pt 1):77-80.

Holte L, Margolis RH, Cavanaugh RM, Jr (1991) Developmental changes in multifrequency tympanograms. *Audiol* 30(1):1-24.

Hunter LL, Feeney MP, Miller JAL, Jeng PS, Bohning S. (2010) Wideband reflectance in newborns: Normative regions and relationship to hearing-screening results. *Ear Hear* 315:599-610.

Hunter LL, Margolis RH. (1992) Multifrequency tympanometry: Current clinical application. *Am J Audiol* 1:33-43.

Hunter LL, Margolis RH. (2011) Middle ear measurement. In: Seewald R, Tharpe A M, (eds). *Comprehensive Handbook of Pediatric Audiology*. San Diego: Plural, 365-388.

Jaffe FB, Hurtado F, Hurtado E. (1970) Tympanic membrane mobility in the newborn (with seven months' follow-up). *Laryngoscope* 80(1):36-48.

Jerger J. (1970) Clinical experience with impedance audiometry. *Arch Otolaryngol* 92(4):311-324.

Joint Committee on Infant Hearing (2007) Joint Committee on infant Hearing. Year 2007 Position statement: Principles and guidelines for early hearing detection and intervention programs. *Pediatrics* 120(4):898-921.

Keefe DH. (2008) Wideband measures of middle-ear functioning. *Asha Leader* 13(1):5-6.

Keefe DH, Bulen JC, Arehart KH, Burns EM. (1993) Ear canal impedance and reflection coefficient in human infants and adults. *J Acoust Soc Am* 94(5):2617-2638.

Keefe DH, Folsom RC, Gorga MP, Vohr BR, Bulen JC, Norton SJ. (2000) Identification of neonatal hearing impairment: Ear-canal measurements of acoustic admittance and reflectance in neonates. *Ear Hear* 21(5):443-461.

Keefe DH, Gorga MP, Neely ST, Zhao F, Vohr B. (2003) Ear-canal acoustic admittance and reflectance measurements in human neonates. II. Predictions of middle-ear dysfunction and sensorineural hearing loss. *J Acoust Soc Am* 113(1):407-422.

Keefe DH, Levi E. (1996) Maturation of the middle and external ears: Acoustic power-based responses and reflectance tympanometry. *Ear Hear* 17(5):361-373.

Kei J, Allison-Levick J, Dockray J, Harrys R, Kirkegard C, Wong J, Maurer M, Hegarty J, Young J, Tudehope D. (2003) High-frequency (1000 Hz) tympanometry in normal neonates. *J Am Acad Audiol* 14(1):20-28.

Kei J, Mazlan R, Seshimo N, Wada H. (2010) Measuring middle ear resonant frequency in neonates: A preliminary study. *XIX National Conference of Audiological Society of Australia*. Sydney.

Kei J, Zhao F. (2012) The challenge of assessing middle ear function in young infants: Summary and future directions. In: Kei J, Zhao F, (eds). *Assessing Middle Ear Function in Infants*. San Diego: Plural, 163-177.

Keith R. (1975) Middle ear function in neonates. *Arch Otolaryngol* 101(6):376-379.

Koike T, Wada H, Kobayashi T. (2002). Modeling of the human middle ear using the finite-element method. *J Acoust Soc Am* 111(3):1306-1317.

Margolis RH, Shanks JE. (1984) Tympanometry. In: Katz J, (ed). *Handbook of Clinical Audiology*. Baltimore, MD: Williams and Wilkins, 438-475.

Margolis RH, Hunter LL. (2000) Acoustic immittance measurements. In: Roeser RJ, Michael V, Hosford-Dunn H, (eds). *Audiology Diagnosis*. New York: Thieme, 381-423.

Margolis RH, Bass-Ringdahl S, Hanks WD, Holte L, Zapala DA. (2003) Tympanometry in newborn infants- 1 kHz norms. *J Am Acad Audiol* 14(7):383-392.

Mazlan R, Kei J, Hickson L, Stapleton C, Grant S, Lim S, Linning R, Gavranich J. (2007) High frequency immitance findings: Newborn vs 6 week old infants. *Int J Audiol* 46(11):711-717.

Mazlan R, Kei J, Hickson L. (2009) Test-retest reliability of the acoustic stapedial test in healthy neonates. *Ear Hear* 30(3):295-301.

Mazlan R, Kei J. (2012) Measuring middle ear function in young infants: An overview. In: Kei J, Zhao F, (eds). *Assessing Middle Ear Functions in Infants*. San Diego: Plural, 17-37.

Meyer SE, Jardine CA, Deverson W. (1997) Developmental changes in tympanometry: A case study. *Br J Audiol* 31(3):189-195.

Murakoshi M, Yoshida N, Sugaya M, et al. (2013) Dynamic characteristics of the middle ear in neonates. *Int J Pediatr Otorhinolaryngol* 77(4): 504-512.

Murakoshi M, Zhao F, Wada H. (2012) Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. In: Kei J, Zhao F, (eds). *Assessing Middle Ear Function in Infants*. San Diego: Plural, 107-129.

Paradise JL, Smith CG, Bluestone CD. (1976) Tympanometric detecion of middle ear effusion in infants and young children. *Pediatrics* 58(2):198-210.

Purdy SC, Williams MJ. (2002) High frequency tympanometry: A valid and reliable immittance test protocol for young infants. *N Z Audiolog Soc Bull* 10:9-24.

Qi L, Liu H, Lutfy J, Funnell WR, Daniel SJ. (2006) A nonlinear finite-element model of the newborn ear canal. *J Acoust Soc Am* 120(6):3789-3798.

Rhodes MC, Margolis RH, Hirsch JE, Napp AP. (1999) Hearing screening in the newborn intesive care nursery: Comparison of methods. *Otolaryngol Head Neck Surg* 120(6):799-808.

Ruah CB, Schachern PA, Zelterman D, Paperella M, Yoon T. (1991) Age related morphologic changes in the human tympanic membrane. A light and electron microscopic study. *Arch Otolaryngol Head Neck Surg* 117(6):627-634.

Sanford CA, Keefe DH, Liu Y-W, Fitzpatrick DF, McCreery RW, Lewis DE, Gorga MP. (2009) Sound-conduction effects on distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry. *Ear Hear* 30(6):635-652.

Shahnaz N. (2008) Wideband reflectance in neonatal intensive care units. *J Am Acad Audiol* 19(5(:419-429.

Sprague BH, Wiley TL, Goldstein R. (1985) Tympanometric and acoustic reflex studies in neonates. *J Speech Lang Hear Res* 28(2):265-272.

Swanepoel DW, Werner S, Hugo R, Louw B, Owen R, Swanepoel A. (2007) High frequency immitance for neonates: A normative study. *Acta Otolaryngol* 127(1):49-56.

Valvik BR, Johnsen M, Laukli E. (1994). Multifrequency tympanometry. Preliminary experiences with a commercially available middle-ear analyzer. *Audiology.* 33(5):245-253.

Vander Werff KR, Prieve BA, Georgantas LM. (2007) Test-retest reliability of wideband reflectance measures in infants under screening and diagnostic test conditions. *Ear Hear* 28(5):669-681.

Wada H, Kobayashi T. (1990) Dynamical behavior of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus. *J Acoust Soc Am* 87(1):237-245.

Wada H, Kobayashi T, Suetake M, Tachizaki H. (1989) Dynamic behaviour of the middle ear based on sweep frequency tympanometry. *Int J Audiol* 28(3):127-134.

Wada H, Kobayashi T, Tachizaki H. (1992) Diagnosis of middle ear disease with eardrum perforation by a newly developed sweep frequency measuring apparatus. *Audiology* 31:(3)132-139.

Wada H, Koike T, Kobayashi T. (1994) The effect of aging on middle ear dynamic characteristics. *J Oto Rhino Laryngol Soc Japan* 97(5):898-904.

Wada H, Koike T, Kobayashi T. (1998) Clinical applicability of the sweep frequency measuring appartus for diagnosis of middle ear diseases. *Ear Hear* 19(3):240-249.

Wada H, Ohyama K, Kobayashi T, Sunaga N, Koike T. (1993) Relationship between evoked otoacoustic emissions and middle-ear dynamic characteristics. *Audiology* 32(5):282-292.

Wada H, Ohyama K, Kobayashi T, Koike T, Noguchi S. (1995) Effect of middle ear on otoacoustic emissions. *Audiology* 34(4):161-176.

Weatherby LA, Bennett MJ. (1980) The neonatal acoustic reflex. *Scand Audiol* 9(2):103-110.

Werner LA, Levi EC, Keefe DH. (2010) Ear-canal wideband acoustic transfer functions of adults and two- to nine-month-old infants. *Ear Hear* 31(5):587-598.

Wilson W. (2012) Anatomy and physiology of the outer and middle ear in young infants. In: Kei J, Zhao F, (eds). *Assessing Middle Ear Function in Infants*. San Diego: Plural, 1-16.

Zhao F, Wada H, Koike T, Ohyama K, Kawase T, Stephens D. (2002) Middle ear dynamic characteristics in patients with otosclerosis. *Ear Hear* 23(2):150-158.

Zhao F, Wada H, Koike T, Ohyama K, Kawase T, Stephens D.(2003) Transient evoked otoacoustic emissions in patients with middle ear disorders. *Int J Audiol* 42(3):117-131.

Chapter 3: Effects of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns

3.1 Background

The dynamic behaviour (acoustic-mechanical properties) of the outer and middle ear in neonates under tympanometric peak pressure conditions was described in the previous chapter. Chapter 3 describes the dynamic behaviour of the outer and middle ear in newborns under pressurised conditions. Unlike adults, the ear canal wall and tympanic membrane of newborns are compliant and flaccid. Hence, the dynamic behaviour of the outer and middle ear in newborns will be different from that in adults.

The present study investigates the dynamic behaviour of the outer and middle ear by inducing positive and negative static pressures (from 200 daPa to -200 daPa, in 50 daPa steps) to the ear canal of newborns. A sample of 122 ears from 86 healthy newborns and a small sample of 10 ears with a conductive condition from 10 newborns were studied. The results of the study provide clinically useful information for differentiating healthy ears from ears with a conductive condition as well as the maturation aspects of the outer and middle ear in newborns.

Chapter 3 of this thesis, entitled, “Effects of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns”, is based on the manuscript published in the *International Journal of Pediatric Otorhinolaryngology*. This published paper is inserted into this thesis with minor modifications. Only the formatting of section sub-headings and numbering of tables and figures have been modified from the original publications to match the thesis format. The referencing format of the paper is retained as per the journal format.

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3.2 Abstract

Objective: The present study investigated the effect of ear canal pressure on the dynamic behaviour of the outer and middle ear in newborns with and without a conductive condition using the sweep frequency impedance (SFI) technology.

Methods: A test battery consisting of automated auditory brainstem response (AABR), transient evoked otoacoustic emission (TEOAE) and 1000-Hz tympanometry (HFT) was performed on 122 ears of 86 healthy newborns and 10 ears of 10 newborns with a conductive condition (failed TEOAE and HFT). The dynamic behaviour of the outer and middle ear, when the pressure applied to the ear canal was varied from 200 to -200 daPa, was evaluated in terms of the sound pressure level (SPL) in the ear canal, resonance frequency (RF) and displacement (ΔSPL).

Results: Application of either a positive or negative static pressure to the ear canal of healthy newborns increased the resonance frequency of the outer (RF1) and middle ear (RF2), but decreased the displacements of the outer (ΔSPL1) and middle ear (ΔSPL2). Positive static pressures resulted in lower SPL while negative static pressures resulted in higher SPL than that at ambient pressure (0 daPa). At -200 daPa, more than 90% of ears showed signs of collapsed ear canal. The dynamic behaviour under various positive and negative static pressures for newborn ears with a conductive condition indicated similar pattern of SPL, RF1 and ΔSPL1 responses for the outer ear as per healthy ears, but abnormal responses for the middle ear.

Conclusions: While both positive and negative pressures applied to the ear canal have the same effect of stiffening the outer and middle ear, negative pressure of up to -200 daPa resulted in more than 90% of ears with a collapsed ear canal. The results of the present study do not only offer useful clinical information for differentiating healthy ears from ears with a conductive condition, but also provide information on the maturation aspects of the outer and middle ear in newborns.

Key Words: Sweep frequency impedance measure, dynamic behaviour, newborns, resonance frequency, middle ear, ear canal.

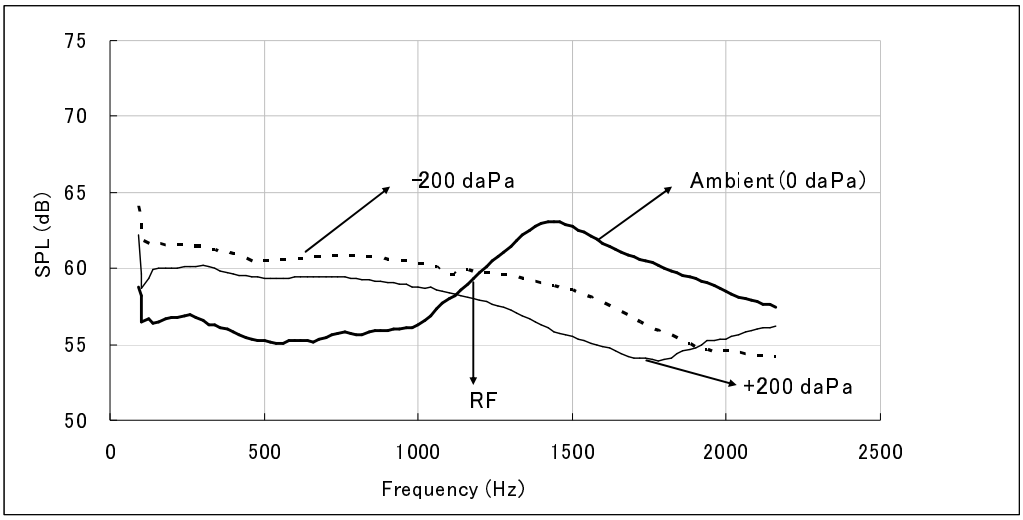
3.3 Introduction

Technological advances have enabled the measurement of acoustical characteristics of the outer and middle ear using multifrequency tympanometry (MFT) [1]. MFT refers to the measurement of middle ear characteristics using tones of more than one frequency. The MFT procedure may either use a sweep frequency technique at multiple applied air pressures to the ear canal or a sweep pressure technique using tone of multiple discreet frequencies [2, 3]. The MFT procedure may also utilise a wideband technique using click stimuli at ambient or multiple applied air pressure to the ear canal [4].

At present, new multi-frequency techniques that measure acoustic-mechanical properties over a wide frequency range have been developed to assess the outer and middle ear function. Two such techniques are sweep frequency impedance (SFI) [1, 5] and wideband acoustic immittance (WAI) [5-8]. The SFI metre, developed by Wada et al. [9], measures the sound pressure in the ear canal while a sweeping tone is presented under various static pressure levels in the ear canal. From the SFI measures, the dynamic behaviour of the outer and middle ear can be described in terms of the sound pressure level (SPL) across frequencies at various static pressure applied to the ear canal. From the SPL results, the resonance frequency (RF) and mobility of the outer and middle ear system (Δ SPL) can be measured [9]. While the SFI is similar in principle to MFT, it does not measure the admittance of the outer and middle ear. Instead, it measures the SPL in the ear canal in dB SPL across the frequencies from 100 to 2200 Hz. The SFI has advantages over the traditional MFT. It is faster than the MFT and it also measures the RF accurately regardless of the direction and rate of change of ear canal pressure. The SFI test has also been reported to be better than the 226-Hz tympanometry in the differential diagnosis of middle ear dysfunction in adults [1, 5, 9-12].

The dynamic behaviour of the outer and middle ear system, as analysed using the SFI metre, of a normally hearing adult is different from that of a healthy newborn (Figure 3.1). The SFI results at ambient pressure (0 daPa) for the adult reveal on inflexion [Figure 3.1 (a)], while the results for the newborn reveal two inflexions [Figure 3.1 (b)]. The differences in dynamic behaviour may be attributed to differences in the anatomy and physiology of the outer/middle ear between the adult and the newborn.

(a)



(b)

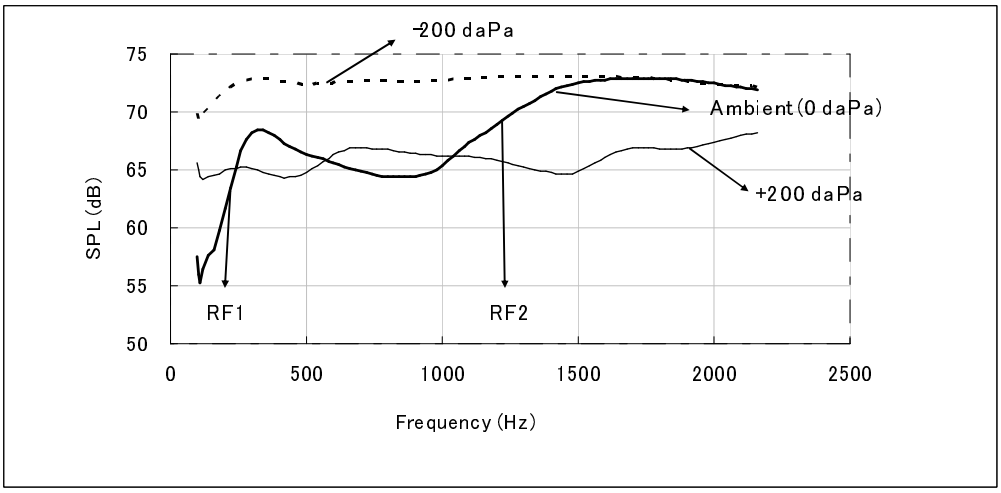


Figure 3.1. SFI results obtained (a) from a normal hearing adult who passed 226 Hz tympanometry. The SPL curve at ambient pressure shows single variation at around 1220 Hz; (b) from a normal hearing newborn who passed HFT and TEOAE. The SPL curve at ambient pressure shows two variations in sound pressure, one (RF1) at around 260 Hz and the second (RF2) at around 1220 Hz. Note: RF = resonance frequency

From an anatomical and physiological perspective, the outer and middle ear system of newborns is not mature at birth [13]. There is a thin layer of elastic cartilage surrounding the entire external auditory canal [14] which makes the ear canal relatively compliant, flaccid and prolapsed [15-17]; newborns have a short ear canal with diameter increasing to 4.4 mm by the age of one month [18] and a short ear canal floor length of 17-22.5 mm and roof length of 11-22.5 mm by age of two month [14]. Orientation of the newborn eardrum is more

horizontal relative to the ear canal axis [18-20]. The middle ear and mastoid cavities are small (452 mm^3) compared to adult tympanic cavity (640 mm^3) [14, 16, 21]. Newborns also have loose ossicular joints [14, 22] which become more stiff with age.

The anatomical and physiological properties of healthy newborns are altered when an external air pressure is applied to the ear canal. On pressurisation, the cartilaginous ear canal diameter increases by an average of 18.3% under positive pressure or decreases by an average of 28.2% of its original value under negative ear canal pressure [23]. Furthermore, ear canal volume changes from 27 to 75% over a range of ± 300 daPa in newborns [22]. In view of these characteristics, the dynamic behaviour of the outer and middle ear of newborns will undoubtedly change in response to pressurisation of the ear canal [15,16,24]. These changes in dynamic behaviour of the outer and middle ear can easily be described using the SFI technique.

While the SFI has been successfully used with children and adults, its application to newborns is relatively new. To date, only two studies have investigated the dynamic behaviour of the outer and middle ear of newborns [25, 26]. In a pilot study, Murakoshi et al. [26] analysed SFI data obtained from 9 neonates under ambient ear canal pressure (0 daPa) condition and found two resonances corresponding to the two inflexions of the sound pressure level (SPL) curve (Figure 3.1b). By comparing their results with that obtained from a gel model which mimicked a newborn ear canal, they showed that the first resonance which occurred at 260 Hz ± 30 Hz, was related to the resonance of the ear canal wall. The second resonance, which occurred at 1130±120 Hz was related to the resonance of the middle ear. Aithal et al. [25] studied the dynamic behaviour of the outer and middle ear of healthy newborns under ambient pressure conditions using a larger sample (N = 100) and reported normative data for the resonance frequencies and Δ SPL (mobility of the system). Their findings were consistent with the results of Murakoshi et al. [26]. Furthermore, they affirmed the feasibility of assessing the function of the outer and middle ear in newborns using the SFI technique.

While the dynamic behaviour of the outer and middle ear in newborns under tympanometric peak pressure condition was described in detail by Murakoshi et al. [26] and Aithal et al. [25], the dynamic behaviour under pressurised conditions has not been systematically investigated. Investigation of the effect of ear canal pressure on the dynamic

behaviour in newborns is important since the ear canal and tympanic membrane of newborns are compliant and flaccid. The present study aimed to investigate the dynamic behaviour of outer and middle ear by inducing positive and negative ear canal pressures in newborn ears. In particular, the study was conducted to address the following questions: (i) Is the dynamic behaviour under pressurised conditions significantly different to that under ambient pressure condition? (ii) Does the dynamic behaviour differ significantly between positive and negative ear canal pressures? (iii) Is the dynamic behaviour under pressurised conditions of a healthy newborn different from that of an ear with a conductive condition?

3.4 Methods

3.4.1. Participants

This study was approved by the Human Research Ethical Committee of Townsville Hospital and Health Service, and the University of Queensland Behavioural and Social Science Ethical Review Committee (Appendix 1). Parents provided written consent for newborns to be included in the study (Appendix 2). The present study included 122 ears from 86 healthy newborns (45 males and 41 females) who passed in a test battery that included automated auditory brainstem response (AABR), transient evoked otoacoustic emission (TEOAE) and high frequency tympanometry (HFT) with a 1000-Hz probe tone. This study sample was a new cohort of newborn ears different from those included in previously published studies [25]. Additionally, 10 ears from 10 newborns who passed the AABR, but did not pass the HFT and TEOAE tests were included to investigate the dynamic behaviour of the outer and middle ear in newborns with a conductive condition.

Table 3.1 shows the mean, standard deviation (SD), and median for gestational age (in weeks), age of testing (in hours) and birth weight (in grams) for 86 healthy newborns. All newborns had uneventful birth history with no risk factors for hearing loss [27].

Table 3.1. Mean, standard deviation (SD), and median of gestational age, age of testing and birth weight for 86 newborns (45 males, 41 females).

	Mean	SD	Median
Gestational age (in weeks)	39.3	1.2	39
Age of testing (in hours)	43.4	18.4	42.1
Birth weight (in grams)	3539.2	425	3555

3.4.2 Procedure

All newborns were tested during their natural sleep or while awake, but quiet and settled. Testing was performed in a quiet room of the maternity ward where ambient noise levels were less than 40 dB A. Hearing screening was performed by trained maternity nursing staff using AABR, while the remaining assessments (HFT, TEOAE and SFI tests) were administered by a clinical audiologist. The entire test battery took an average of 30 min for both ears for a well settled newborn. Wherever possible, the HFT and TEOAE tests were completed for both ears of each newborn with no particular test order. The most accessible ear was tested first. SFI results were analysed independent of HFT and TEOAE results i.e., the audiologist who analysed SFI result was not involved in the classification of HFT and TEOAE results.

The present study used a test battery approach (pass in AABR, TEOAE and HFT) as the reference standard for normal middle ear function. Although a pass in TEOAE or HFT or AABR does not always rule out middle ear dysfunction [28-30], a pass in all three tests constituted a more stringent "reference" standard than a single test reference standard.

3.4.2.1. Automated Auditory Brainstem Response (AABR)

AABR screening was always performed first as part of the state mandated universal newborn hearing screening (UNHS) program using an ALGO3 newborn hearing screener (Natus Medical Inc.). Clicks were presented at 35 dB nHL to both ears simultaneously during testing. A pass or refer result for each ear was automatically recorded by the equipment. Passing the AABR screen was necessary to ensure likelihood of grossly normal auditory function.

3.4.2.2 High frequency tympanometry (HFT)

HFT was performed using a Madsen Otoflex 100 acoustic immittance device (GN Otometrics) with a 1000-Hz probe tone. Admittance (Ya) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec. Pass criteria were a single positively peaked tympanogram with the middle ear pressure between 50 and -150 daPa and peak compensated static admittance (Ypc) (+200 daPa tail to peak) of at least 0.2 mmho [31].

3.4.2.3 Transient Evoked Otoacoustic Emissions (TEOAE)

TEOAE test was performed using a Scout sport system (Biologic Navigator Plus). The signal consisted of wideband clicks of 80 µs duration delivered at 80 dB pkSPL to the ear via a probe. Emissions were measured at 2000, 3000 and 4000 Hz. Pass criteria included a reproducibility of 70% and difference of at least 3 dB between the amplitude of the emissions and associated noise floor in one-third octave frequency bands from 2000 to 4000 Hz [30]. This "Pass" criteria was based on study by Kei et al. (2003) who used same criteria for development of normative data for HF tympanometry (1000 Hz) in neonates (30).

3.4.2.4 Sweep Frequency Impedance (SFI) test

SFI test was performed using a new SFI unit developed for testing newborns [26]. A full description of SFI unit used in testing infants is provided elsewhere [25, 26], however a brief description is provided here (Figure 3.2). The SFI device consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, an air pump, a pressure sensor, and a pressure relief valve. The new probe used for testing newborns is small with a diameter of approximately 3 mm. The new probe consists of three tubes: the first tube to apply static pressure (Ps) to the ear canal, the second tube to deliver sound to the external ear canal via an earphone, and the third tube to measure sound pressure in the external ear canal using a microphone. A specially designed cuff suitable for testing neonates is attached to the tip of the probe to obtain a hermetic seal during testing. This new SFI unit, controlled using LabView under MS WINDOWS, also performs HFT first as part of the automated test procedure. However, the HFT results were not included in the analyses because a commercially available Madsen Otoflex 100 device was used instead.

After performing the HFT, the SFI test began by presenting a probe tone with frequency sweeping from 100 to 2200 Hz while the external auditory canal static pressure (Ps) was held constant at 200 daPa. This measurement was repeated with Ps reduced in 50

daPa steps down to -200 daPa. The entire automated SFI procedure took less than one minute to complete the test in each ear. The sweeping probe tone level was kept below 75 dB SPL to reduce the possibility of eliciting an acoustic stapedial reflex. The SFI results measured at multiple static pressures provide a comprehensive three-dimensional view (SPL-frequency-static ear canal pressure) of the dynamic behaviour of the outer and middle ear.

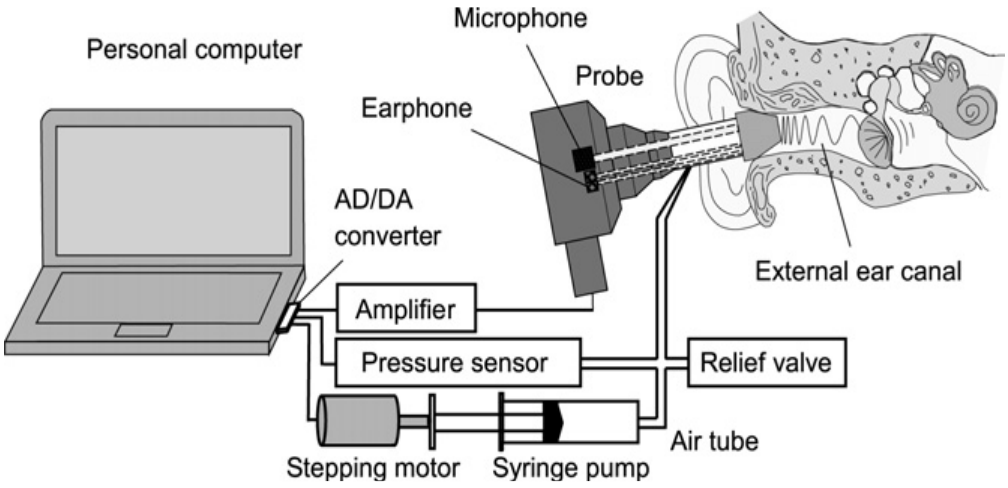


Figure 3.2. Block diagram of SFI metre used to test newborns in this study. The SFI metre consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, a syringe pump, a pressure sensor and a relief valve. This new unit is controlled using LabView under WINDOWS. [From Murakoshi et al (2013). Int J Pediatr Otorhinolaryngol. Copyright © 2012 by Elsevier Ireland Ltd. Reprinted with permission of Elsevier Ireland Ltd.]

3.5 Results

Figure 3.3 shows typical SFI results obtained from the left ear of a healthy one-day-old newborn who passed AABR, HFT and TEOAE tests. For purpose of clarity, only traces obtained at 0, +200 and -200 daPa are shown. At ambient pressure, (Ps = 0 daPa), the graph (bold SPL curve) shows two resonances at frequencies between F_b1 (130 Hz) and F_a1 (350 Hz) and between F_b2 (900 Hz) and F_a2 (1560 Hz). Previous studies have shown that the greatest variation (volume displacement) of SPL (ΔSPL) occurs at median frequencies RF1 and RF2, which are halfway between the frequencies F_a1 and F_b1, and between F_a2 and F_b2, respectively [9, 26]. The first resonance frequency (RF1) is defined as the frequency at which the SPL varies considerably between F_b1 to F_a1. Hence, RF1 and the corresponding change in SPL (Δ SPL1) are defined as shown in Equations (3.1) and (3.2) [9, 26].

First Resonance Frequency, RF1 = $(F_a1+F_b1) / 2$ Equation (3.1)

SPL change at RF1, $\Delta SPL1 = P_a1 - P_b1$ Equation (3.2)

Similarly, the second resonance frequency (RF2) and the corresponding change in SPL (Δ SPL2) are defined as shown in Equations (3.3) and (3.4).

Second Resonance Frequency, RF2 = $(F_a2+F_b2) / 2$ Equation (3.3)

SPL change at RF2, $\Delta SPL2 = P_a2 - P_b2$ Equation (3.4)

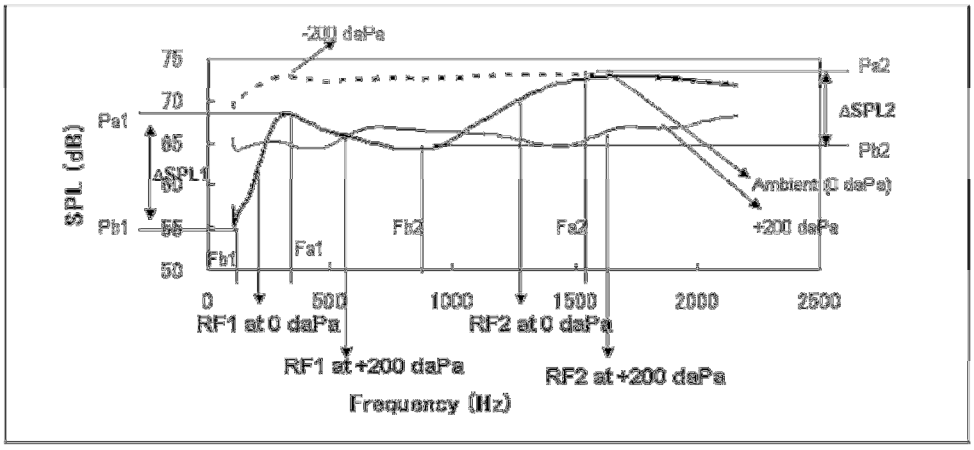


Figure 3.3. SFI results obtained from a healthy 2-day-old newborn who passed the test battery. The static ear canal pressure (daPa) applied were +200, 0 (ambient pressure), and -200 daPa. P_a1 and P_b1 are the maximum and minimum sound pressures, and F_a1 and F_b1 are the frequencies corresponding to these sound pressures (first variation). P_a2 and P_b2 are the maximum and minimum sound pressures, and F_a2 and F_b2 are the frequencies corresponding to these sound pressures (second variation). RF1 and RF2 are defined by $(F_a1+F_b1)/2$, and $(F_a2+F_b2)/2$, respectively. ΔSPL1 and ΔSPL2 are defined by $(P_a1- P_b1)$ and (P_a2-P_b2), respectively. Figure also shows an increase in RF1 and RF2 when a pressure of +200 daPa was applied to the ear canal.

As illustrated in the Figure 3.3, when the static pressure (Ps) = 0 daPa (ambient pressure), RF1 = 240 Hz, Δ SPL1 = 13 dB, RF2 = 1230 Hz and Δ SPL2 = 8 dB. These results indicate that there are two distinct resonance frequencies at which the SPL varies considerably. The Δ SPL variation reflects the mobility of the outer and middle ear system at these frequencies [25, 26]. According to Murakoshi et al. [26] and Aithal et al. [25], RF1 and

ΔSPL1 are associated with resonance in the outer ear, while RF2 and ΔSPL2 are associated with resonance in the middle ear.

Figure 3.4 shows effect of static ear canal pressure (Ps) on SFI measure obtained from a newborn at different static pressure levels. Although SFI results were recorded at 50 daPa intervals starting from +200 daPa to -200 daPa, SFI data (RF and ΔSPL) were analysed only for static ear canal pressures at +200 daPa, +100 daPa, +50 daPa, 0 daPa, -50 daPa, -100 daPa and -200 daPa. The dynamic behaviour altered as the ear canal pressure was increased from 0 to + 200 daPa. In particular, when the static pressure was increased from 0 daPa to +50 daPa, the SPL decreased considerably between 1000 Hz and 2000 Hz, while the SPL at the lower frequencies increased slightly. When compared with the SFI data at 0 daPa, both RF1 and RF2 increased (i.e., shifted towards higher frequencies) while ΔSPL1 and ΔSPL2 decreased. Further increase in static pressure to +100 and +200 daPa led to reduced SPL level between 1000 Hz and 2000 Hz, as well as increased RF1 and RF2, and decreased ΔSPL1 and ΔSPL2 values. This change in dynamic behaviour with increased ear canal pressure is typical of healthy newborn ears.

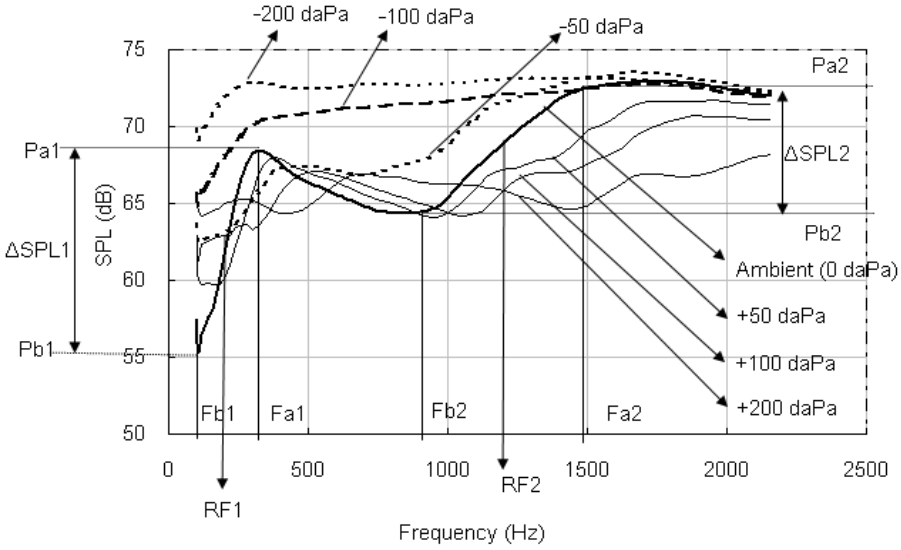


Figure 3.4. SFI measures at different static ear canal pressures (Ps). The static ear canal pressures (daPa) applied were +200, +100, +50, 0 (ambient), -50, -100, and -200 daPa. P_a1 and P_b1 are the maximum and minimum sound pressures, and F_a1 and F_b1 are the frequencies corresponding to these sound pressures (first variation) at 0 daPa. P_a2 and P_b2 are the

maximum and minimum sound pressures, and F_a2 and F_b2 are the frequencies corresponding to these sound pressures (second variation) at 0 daPa.

When the static pressure was decreased from 0 daPa to -50 daPa, the SPL between 500 Hz and 1500 Hz increased considerably while the SPL remained unchanged between 1500 Hz and 2200 Hz. RF1 increased considerably and RF2 decreased slightly, while ΔSPL1 decreased considerably and Δ SPL2 remained practically unchanged. This change in dynamic behaviour indicates that a mild negative pressure (-50 daPa) had greater influence on RF1 and ΔSPL1 than on RF2 and ΔSPL2. When a static negative pressure of -100 daPa was applied to the ear canal, the SPL between 200 Hz and 1500 Hz increased further with practically no change in SPL beyond 1500 Hz. The morphology of the SPL curve changed significantly without much variation of SPL with frequency (a relatively flat response across frequencies), suggesting that the ear canal had collapsed [25, 26]. Hence, no RF or ΔSPL could be measured. Further decrease of static pressure to -200 daPa showed similar flat responses with greater overall SPL than that obtained at -100 daPa. In the present study sample of 122 ears of healthy newborns, there were 110 ears collapsed at -200 daPa, 53 ears at -100 daPa, and 6 ears at - 50 daPa.

Table 3.2 shows the number of ears, mean, SD, median for RF1, RF2, ΔSPL1, and ΔSPL2 at different static ear canal pressures in newborns. At ambient pressure, mean RF2 (1243 Hz) was 4.3 times larger than mean RF1 (287 Hz), and overall mean ΔSPL1 (8.2.dB) was 1.7 times larger than mean ΔSPL2 (5 dB). These results are consistent with previous published studies [25, 26]. The SFI results at positive pressures showed a trend of increasing RF1 and RF2, and decreasing ΔSPL1 and ΔSPL2 with increasing pressure up to +200 daPa. The SFI results at negative pressures showed a clear trend of increasing RF1 and decreasing ΔSPL1 with decreasing (more negative) pressure. However, this trend is not evident for RF2 and ΔSPL2.

Table 3.2. Mean, standard deviation (SD) and median of RF1, RF2, Δ SPL1, Δ SPL2 at different static ear canal pressure levels for 86 newborns with normal middle ear condition (Note: At -50 daPa, n=116 as 6 ear canals collapsed; at -100 daPa, n=69 as 53 ear canals collapsed and at -200 daPa, n=12 as 110 ear canals collapsed) and 10 newborns with conductive condition (Note: n = 10 ears).

		RF1 (Hz)			ΔSPL1 (dB)		
Pressure (daPa)	N (ears)	Mean	SD	Median	Mean	SD	Median
Normal condition							
-200	12	593	59	600	3.6	1.6	3.3
-100	69	442	102	440	4.4	1.7	4.3
-50	116	363	105	350	6	2.8	5.7
0	122	287	71	260	8.2	3.2	8
50	122	377	96	360	5.9	2.4	5.5
100	122	454	107	469	4.6	2.1	4
200	122	589	106	600	2.8	1.1	2.8
Conductive condition							
-200	10	698	78	724	3.2	1.9	3.1
-100	10	558	127	595	6.3	2.4	7.2
-50	10	406	130	444	7.3	2.6	7.7
0	10	280	77	270	10.2	3.1	10.1
50	10	367	125	389	8.5	3.2	8.8
100	10	489	134	520	7.5	3.0	7.4
200	10	647	74	620	5	2.1	5.4

		RF2 (Hz)			ΔSPL2 (dB)		
Pressure (daPa)	N (ears)	Mean	SD	Median	Mean	SD	Median
Normal condition							
-200	12	1438	215	1444	1.8	1.4	1.1
-100	69	1198	202	1180	4	1.8	4.1
-50	116	1230	220	1230	4.9	2.1	4.8
0	122	1243	211	1245	5	2.3	5
50	122	1345	226	1335	5.2	2.4	5.5
100	122	1496	235	1475	4.6	2.3	4.5
200	122	1681	297	1685	4	2.6	3.5
Conductive condition							
			Absent			Absent	

To investigate the effect of static ear canal pressure on RF and ΔSPL, a general linear model univariate analysis of variance (ANOVA) was applied separately to the RF1, RF2, ΔSPL1 and ΔSPL2 data with static pressure in the ear canal (-200 daPa, -100 daPa, -50 daPa, 0 daPa, 50 daPa, 100 daPa and 200 daPa) as independent (fixed) factors. The effect of static pressure in the ear canal was significant for RF1 [F (6, 678) = 118.3, p = 0.00], RF2 [F (6, 678) = 60, p = 0.00], ΔSPL1 [F(6, 678) = 37.6, p = 0.00] and ΔSPL2 [F(96,678) = 8.1, p = 0.00], with RF values significantly increased. However, ΔSPL values significantly reduced with the introduction of both positive and negative static pressure in the ear canal. The magnitude of effect (partial eta squared) was large for RF1 (0.51), RF2 (0.35), and ΔSPL1 (0.25), and effect was medium for ΔSPL2 (0.07).

To further investigate the effect of static ear canal pressure on resonance frequency and mobility of ear drum, post hoc multiple pair-wise comparison tests with Bonferroni adjustment were performed on the SFI data. Table 3.3 shows results comparing the SFI measures at 0 daPa (ambient) with those at other ear canal pressures (50 daPa, 100 daPa, 200 daPa, -50 daPa, -100 daPa, and -200 daPa). RF1 was significantly different at all pressure levels whereas RF2 was significantly different only at positive ear canal pressure (0 daPa vs. 50 daPa, 0daPa vs. 100 daPa, and 0daPa vs 200 daPa). The ΔSPL1 was significantly different at all pressure levels, whereas ΔSPL2 was significantly different only extreme pressure levels (0 daPa vs +200 daPa and 0 daPa vs -200 daPa).

Table 3.3. Pair wise comparisons: Results of post hoc analysis with Bonferroni adjustment for SFI measures at different static ear canal pressures (daPa) relative to ambient pressure.

	0 Vs 50	0 Vs 100	0 Vs 200	0 Vs -50	0 Vs -100	0 Vs -200
RF1	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
RF2	0.02*	0.00*	0.00*	1.00	1.00	0.14
ΔSPL1	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
ΔSPL2	1.00	1.00	0.01*	1.00	0.08	0.00*

*indication of statistical significance, p<0.005.

With the application of positive pressures to the ear canal, there was a shift in RF1 and RF2 towards higher frequencies. The shift was significant for all static ear canal pressure

levels for RF1 relative to ambient pressure. However, RF2 showed a significant shift to higher frequencies only at positive pressure levels (50 daPa, 100 daPa, and 200 daPa) relative to ambient pressure. When the static ear canal pressure reached +200 daPa, mean RF1 doubled (2.1 times larger) relative to that at ambient pressure, and mean RF2 increased 1.4 times when compared to that at ambient pressure. With the introduction of – 200 daPa negative static ear canal pressure, mean RF1 significantly increased with respect to ambient pressure (287-593 Hz), whereas RF2 did not show any significant change.

As a measure of inter-subject variability, SD for SFI measures was calculated. With the exception of 200 daPa for RF2, SDs generally remained constant across all static pressure levels. The increased SD for RF2 at 200 daPa could be due to small sample size as it included only 12 ears. As the ear canal pressure changed from positive to negative, ΔSPL1 showed greater changes than Δ SPL2. As the static pressure varied from 0 to -100 daPa, SD for Δ SPL2 decreased slightly, whereas SD for Δ SPL1 decreased considerably. As the ear canal pressure changed from ambient to positive pressure, ΔSPL1 showed a sharp decrease whereas ΔSPL2 remained steady. Overall, ΔSPL1 showed more variability than ΔSPL2.

Table 3.4 shows the number and percentage of ears collapsed at different static ear canal pressure levels. About 4.9 %, 38.5% and 40.2% of the ear canals collapsed when static ear canal pressure reached -50 daPa, -100 daPa, and -150 daPa, respectively. Cumulatively, 83.6% and 90.2% of ear canals collapsed when static ear canal pressure reached -150 daPa and -200 daPa, respectively.

Table 3.4. Total number (n) and percentage (%) of ear canals collapsed at different negative static ear canal pressures (daPa) relative to ambient pressure (0 daPa)

Static ear canal pressure (daPa) relative to ambient pressure	Number of ears (n)	Collapse (%)	Cumulative (%)
-50	6	4.9	4.9
-100	47	38.5	43.4
-150	49	40.2	83.6
-200	8	6.6	90.2
No collapse	12	9.8	100

Figure 3.5 illustrates the SFI results obtained from a newborn who passed the AABR but did not pass the HFT and TEOAE tests. In the present study, this newborn was considered to have a conductive dysfunction. At ambient pressure, the first variation in sound pressure (RF1) was observed at around 400 Hz with ΔSPL1 of 8 dB. However, the second variation (RF2) was absent. With increasing positive ear canal pressures, SPL between 700 Hz and 2200 Hz decreased progressively from 70 to 66 dB at 2200 Hz. In contrast, SPL increased considerably between 500 and 1500 Hz as the static pressure changed from -50 to -200 daPa.

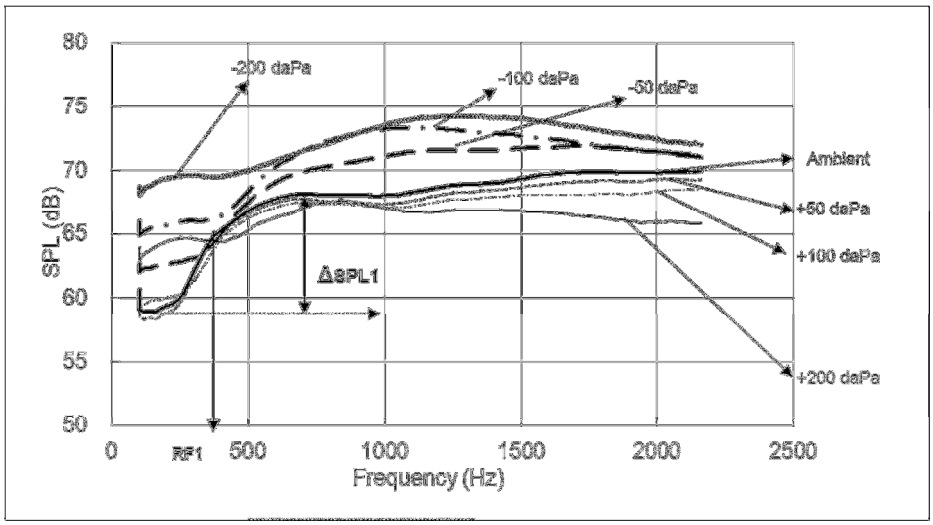


Figure 3.5. SFI results obtained from the right ear of a one-day-old newborn who passed AABR but did not pass HFT and TEOAE. The static ear canal pressures (daPa) applied were +200, +100, +50, 0 (ambient pressure), -50, -100, and -200 daPa.

To investigate the effect of static ear canal pressure on SFI results in newborns with a conductive condition (did not pass the HFT and TEOAE tests), data from 10 ears were analysed. Table 3.2 shows mean RF1and mean ΔSPL1 for ears with a conductive and normal condition. Results reveal that there is a trend of increasing RF1 and decreasing ΔSPL1 with changes in static pressure in the ear canal for healthy ears and ears with a conductive condition. However, RF2 and SPL2 were absent in ears with conductive condition.

3.6 Discussion

The present study investigated the effect of ear canal pressure on the dynamic behaviour of the outer and middle ear in newborns using the SFI technology. The results

demonstrated that the dynamic behaviour of the outer and middle ear changed significantly between ambient pressure and pressurised conditions. There were also significant differences between effects of positive and negative ear canal pressures.

3.6.1 Effect of positive ear canal pressure on dynamic behaviour

As shown in Figure 3.4, SFI results obtained at ambient pressure show two regions of resonance, with the first resonance (of the outer ear) occurring at RF1 and second resonance (of the middle ear) occurring at RF2. The mean RF1 for this cohort of normal newborns was 287 ± 71 Hz, while the mean RF2 was 1243 ± 211 Hz (Table 3.2). These results are consistent with the results of previous studies [25, 26].

When the static pressure was increased from 0 to +50, +100 and +200 daPa, the corresponding SPL curves were progressively lower especially in the 1000-2200 Hz region, indicating an overall decrease in SPL. This phenomenon occurred because the increase in static pressure would have distorted the ear canal and increased the ear canal volume in newborns [22], resulting in an overall decrease in SPL across the frequencies. Moreover, with increasing positive pressure, the SPL decreased progressively at frequencies between 1000 Hz and 2000 Hz, while the SPL at the lower frequencies increased slightly and steadily. Incidentally, Sanford and Feeney [24] found a similar pressure effect using wideband acoustic immittance measures. They applied positive pressure to the ear canal of 4-week-old infants and found that wideband reflectance increased in the low frequencies but decreased in the high frequencies relative to the reflectance results obtained at ambient pressure. They concluded that increased positive pressure increased the stiffness of the ear canal walls. They also found that the change in reflectance with increasing positive pressure in older infants (≥ 12 months) was smaller than that in 4-month-old infants, indicating maturation of the outer and middle ear with age.

A close examination of the dynamic behaviour showed that when positive pressures up to +200 daPa were applied to the ear canal, RF1 and RF2 increased (Figure 3.4), while both ΔSPL1 and ΔSPL2 decreased. These changes may be attributed to the increased stiffness of ear canal wall due to increased strain on the inferior wall of the ear canal and the tympanic membrane by the static ear canal pressures [26].

3.6.2 Effect of negative ear canal pressure on dynamic behaviour

When the static pressure was decreased from 0 daPa to -50 daPa, the SPL between 500 Hz and 1500 Hz increased considerably while the SPL remained unchanged between 1500 Hz and 2200 Hz (Figure 3.4). There was an increase in overall SPL which may be caused by a smaller ear canal volume induced by the negative pressure. The SPL curve obtained at a pressure of -50 daPa showed two inflexions corresponding to two resonances. Surprisingly, RF1 increased considerably and RF2 decreased slightly, while ΔSPL1 decreased considerably and ΔSPL2 remained practically unchanged. This change in dynamic behaviour indicates that the negative pressure produced greater effect on the outer ear than on the middle ear.

When a static negative pressure of -100 daPa was applied to the ear canal, the SPL increased further in the low frequencies (200-1500 Hz) with practically no change in SPL beyond 1500 Hz (Figure 3.4). The SPL curve changed from a curve with two inflexions to a relatively flat curve, suggesting that the ear canal had collapsed [25, 26] and no RF or ΔSPL could be measured. This SPL pattern was observed in 53 out of 122 ears of healthy newborns. For the 69 ears that did not show this flat pattern, RF1 continued to increase and ΔSPL1 decreased while RF2 decreased slightly and ΔSPL2 decreased slightly. In general, these results indicate that the stiffness of the outer and middle ear system continued to increase with further decrease in static pressure.

Further decrease of static pressure to -200 daPa resulted in flat SPL responses in 110 out of 122 ears, indicating collapsed ear canal in these ears (Figure 3.4 & Table 3.2). At this pressure, the overall SPL increased more than that at -100 daPa due to further decrease in ear canal volume and greater stiffness of the collapsed ear canal wall. For the 12 ears that showed non-flat SPL responses, RF1 and RF2 increased considerably while ΔSPL1 and ΔSPL2 continued to decrease.

3.6.3 Acoustic-mechanical properties of the outer and middle ear in healthy newborns

The dynamic behaviour is dependent on the acoustic-mechanical properties of the outer and middle ear system in response to sound stimulation under ambient or pressurised conditions. The SFI results showed that application of positive or negative static ear canal pressure resulted in significant increase in RF1 and decrease in ΔSPL1 (Table 3.2 and Figure 3.4), indicating that the vibration of the ear canal walls was reduced as the compliant ear

canal became stiffer. Wada et al. reported the relationship between the stress and strain of the cartilage of the inferior wall of the external ear canal [32]. They noted that the slope of stress-strain curve increased nonlinearly. Since the slope is equivalent to Young's modulus, the increase in the slope leads to an increase in the stiffness of the ear canal wall. Hence, when the ear canal was pressurised, the strain of the inferior wall of the ear canal could have increased, leading to an increase in the stiffness of the ear canal wall and resulting in higher RF1 and smaller ΔSPL1 [26]. Such pressure-related effects have also been observed by Sanford and Feeney [24] who remarked that the compliant energy-absorbing ear canal walls in young infants became stiffer with the introduction of an external static pressure.

The SFI results showed that application of positive or negative static ear canal pressure also resulted in an increase in RF2 and decrease in ΔSPL2. When compared to the results obtained at ambient pressure, the changes in RF2 and ΔSPL2 were small for static pressures up to ±100 daPa. However, these results suggest that pressurizing the ear canal produced less impact on the acoustic-mechanical properties of the middle ear than on the outer ear.

Further observation of the impact of static pressure on the volume displacements (ΔSPL1 and ΔSPL2) of the outer and middle ear revealed differential effects depending on the direction of the pressure change. For example, mean ΔSPL1 decreased progressively from 8.2 dB at ambient pressure to 2.8 dB at +200 daPa and 3.6 dB at -200 daPa (Table 3.2). This finding suggests that positive (+200 daPa) static pressure produced a greater impact than negative pressure (-200 daPa) on the mobility of the outer ear. This observation is supported by a three-dimensional nonlinear finite-element model study of a 22-day-old neonate [22]. In this study, Qi et. al [22], reported that displacements of ear canal wall are slightly larger under positive pressures than under negative pressures.

In contrast, mean ΔSPL2 decreased progressively from 5.0 dB at ambient pressure to 4.0 dB at +200 daPa and 1.8 dB at -200 daPa (Table 3.2). This finding suggests that positive (+200 daPa) static pressure produced a smaller effect than negative pressure (-200 daPa) on the mobility of the middle ear. This observation is consistent with the findings of Qi et al. study using a nonlinear finite element model of the newborn middle ear [33]. Qi and colleagues [33] reported larger displacement of the TM for negative pressures than positive pressures. From a clinical perspective, the difference in the pattern of change between ΔSPL1

and ΔSPL2 revealed the differential effects of ear canal pressure on the outer ear and middle ear, respectively.

3.6.4 Effect of static ear canal pressure on newborn ears with a conductive condition

Figure 3.5 shows the SFI results obtained from the right ear of a one-day-old newborn who passed AABR, but did not pass HFT and TEOAE, indicating the possibility of a conductive condition [34]. The SPL curve at ambient pressure showed only one inflexion with RF1 at 375 Hz and ΔSPL1 of 8 dB. However, the second inflexion in SPL curve was absent, indicating dysfunction in the middle ear. Further examination of the results showed that with increasing positive pressure to +200 daPa, the SPL varied between 66 to 70 dB. When negative pressures from -50 to -200 daPa were applied, the SPL increased significantly and progressively to 74 dB. The possibility of a collapsed ear canal at pressures from -100 to -200 daPa cannot be excluded in view of the high SPL level [26]. These SFI results represent the typical response pattern of an ear with a conductive condition.

In order to depict the dynamic behaviour of the outer and middle ear system for ears with and without conductive condition at the group level, the SFI results of the 10 ears that did not pass HFT and TEOAE were averaged and compared with normal group who passed AABR, HFT and TEOAE (Table 3.2). Overall, RF1 increased and ΔSPL1 decreased as static ear canal pressure was either increased or decreased for both groups. However, the results of static pressure on the dynamic behaviour of the middle ear in ears with a conductive condition showed a distinctive pattern from that in healthy ears (Figure 3.5). None of the SPL curves corresponding to static pressures of -100, -50, 0, +50, +100 and +200 daPa showed any inflexion (resonance) in the frequency region between 1000 and 2000 Hz, clearly showing absence of RF2. This pattern is clinically significant as it can potentially identify ears with a conductive condition.

Although RF2 decreased for negative middle ear pressures (-50 and -100 daPa), it increased for -200 daPa, (i.e., middle ear became more stiff) (see Table 3.2). The reason for this unexpected result is unknown. It may be due to normal variations in the measurements. Further analysis showed no statistical significance in the mean RF2 at -50 or -100 daPa when compared to that at 0daPa (Table 3.3). The collapse of ear canal in newborns can easily contaminate the measurement of RF2 and the reported low resonance in conductive hearing

loss may be due to ear canal wall movements rather than decrease in middle ear resonance (16, 17).

3.6.5 Collapse of ear canal at static negative pressures

As shown in Table 3.4, the number of ears with collapsed ear canal increased with increasing static ear canal pressure. About 4.9 percent of ears had this condition even at a mild negative pressure of -50 daPa, indicating that ears with very flaccid ear canal walls could collapse at this pressure. At ear canal pressures of -100 and -150 daPa, the proportion of ears with collapsing ear canal conditions increased to 38.5% and 40.2%, respectively, indicating that the flaccid ear canal walls in newborns are sensitive to negative ear canal pressures. Only 12 ears did not show any evidence of collapsed ear canal at a pressure of -200 daPa. These results provide further evidence that the ear canal walls of newborns are usually highly compliant and that the inferior wall elastic cartilage in the ear canal may be deformed when a negative pressure of about -200 daPa was applied [15, 16, 19, 22, 23, 35]. It can be predicted that older infants, who have less compliant ear canal walls, would have a smaller proportion of ears with a collapsed ear canal condition than newborns at -200 daPa. Hence, these results of collapsed ear canals at negative ear canal pressures may provide information on the maturation progress of the infant ears.

In a recent study using gel model, Murakoshi et al. [26] showed that infant ear canal started collapsing when a significant negative pressure was applied to the ear canal. At -200 daPa, the ear canal behaves like a 5 mm calibration cavity. They noted that a neonate's ear canal probably collapsed at about 5 mm from the probe tip by application of negative pressure, resulting in a similar response obtained in the 5 mm calibration cavity. Clinically, these results imply that tympanometric procedures on newborns should not apply negative ear canal pressures beyond -200 daPa because of the collapsed ear canal conditions. The collapsing ear canal in newborns due to negative static ear canal pressure beyond -200 daPa would render the measurement of peak compensated static admittance using the negative tail compensation method unreliable.

The present study supports the theory that compliant and flaccid infant ear canal easily collapses for negative ear canal pressure and expands for positive ear canal pressure, thereby changing the dynamic behaviour of the outer and middle ear.

3.6.6 Limitations

Although the SFI test was automated to perform HFT and SFI smoothly, it required multiple pressurizations as a sweeping frequency tone was delivered to the ear. While the pressurisation might be a source of discomfort for newborns, the SFI test required a tight probe seal for repeated sweeps. At times, it was difficult to maintain a hermetic seal for the entire test for some newborns. This difficulty was partially overcome by testing newborns when they were asleep.

Additionally, use of multiple probe tips for conducting HFT, TEOAE and SFI tests in the present study disturbed some newborns. Testing had to be discontinued for some newborns who became unsettled due to multiple probe insertion. Improvement in instrumentation to include a single probe assembly to perform multiple tests is desired. This improvement will reduce overall testing time and increased completion rate in testing newborns.

Another limitation of the present study is related to the lack of "gold standard" for confirmation of middle ear status in newborns. A pass in AABR does not rule out subtle middle ear dysfunction [28, 30]. Similarly, a pass in HFT or TEOAE test alone does not guarantee normal middle ear function, as infants and children with subtle middle ear dysfunction can pass this test [29, 36]. While the use of single test alone may not be accurate, use of battery of tests may provide greater assurance of an efficient conductive pathway in newborns. Hence, a test battery approach was used in the present study to evaluate the middle ear status [37]. However, it is acknowledged that the test battery reference standard is not an ideal "gold standard" for detecting conductive conditions.

3.7 Conclusions

The present study found that applying positive or negative pressure to the ear canal of healthy newborns increased the RF1 and RF2, but decreased ΔSPL1 and ΔSPL2. The dynamic behaviour of the outer and middle ear under positive pressures was distinctively different to that under negative pressures. More than 83% of ears showed evidence of collapse when the static pressure was decreased to -150 daPa. Furthermore, the effect of ear canal pressure on the outer and middle ear of newborns with a conductive condition showed a different pattern of results from that of healthy newborns, suggesting that the dynamic

behaviour observed under various static ear canal pressures can provide additional clinical information for differentiating healthy ears from ears with a conductive condition.

3.8 Acknowledgements

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3.9 References

[1] F. Zhao, J. Wang, Assessing middle ear function in humans using multifrequency tympanometry: An overview. In: J. Kei, F. Zhao. F (eds), Assessing middle ear function in infants, Plural, San Diego, 2012, pp. 87-106.

[2] R. H. Margolis, K. J. Van Camp, R.H. Wilson, W. L. Creten, Multifrequency tympanometry in normal ears, Audiology. 24 (1985) 44-53.

[3] R. H. Margolis, M. V. Goycoolea, Multifrequency tympanometry in normal adults, Ear Hear. 14 (1993), 408-413.

[4] D. H. Keefe, R. Ling, J. C. Bulen, Method to measure acoustic impedance and reflection coefficient, J. Acoust. Soc. Am. 91(1992) 470-485.

[5] M. Murakoshi, F. Zhao, H. Wada, Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. In: J. Kei, F. Zhao (Eds). Assessing middle ear function in infans, Plural, San Diego, 2012; 107-129.

[6] L. L. Hunter, B. A. Prieve, J. Kei, C.A. Sanford, Pediatric applications of wideband acoustic immittance measures, Ear Hear. 34 (2013) 36S-42S.

[7] J. Kei, C. A. Sanford, B. A. Prieve, L. L. Hunter, Wideband acoustic immittance measures: developmental characteristics (0 to 12 months), Ear Hear. 34 (2013) 17S-26S.

[8] D. H. Keefe, R. C. Folsom, M. P. Gorga, B. R. Vohr, J. C. Bulen, S. J. Norton, Identification of neonatal hearing impairment: ear-canal measurements of acoustic admittance and reflectance in neonates, Ear Hear. 21 (2000) 443-461.

[9] H. Wada, T. Kobayashi, M. Suetake, H. Tachizaki, Dynamic behaviour of the middle ear based on sweep frequency tympanometry, Audiology 28 (1989) 127-134.

[10] H. Wada, T. Kobayashi, Dynamical behavior of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus, J. Acoust. Soc. Am. 87 (1990) 237-245.

[11] H. Wada, T. Koike, T. Kobayashi, Clinical applicability of the sweep frequency measuring appartus for diagnosis of middle ear diseases, Ear Hear. 19 (1998) 240-249.

[12] F. Zhao, H. Wada, T. Koike, K. Ohyama, T. Kawase, D. Stephens, Middle ear dynamic characteristics in patients with otosclerosis, Ear Hear. 23 (2002) 150-158.

[13] W. Wilson, Anatomy and physiology of the outer and middle ear in young infants. In: J. Kei, F. Zhao. F (eds), Assessing middle ear function in infans, Plural, San Diego, 2012, pp.1-

[14] M.S. McLellan, C. H. Webb, Ear studies in the newborn infant, J. Pediatr. 51 (1957) 672-677.

[15] L. Holte, R. H. Margolis, R. M. Cavanaugh, Developmental changes in multifrequency tympanograms, Audiology 30 (1991) 1-24.

[16] D. H. Keefe, J. C. Bulen, K. H. Arehart, E. M. Burns, Ear canal impedance and reflection coefficient in human infants and adults, J. Acoust. Soc. Am. 94 (1993) 2617-2638.

[17] B. H. Sprague, T. L. Wiley, R. Goldstein, Tympanometric and acoustic reflex studies in neonates, J. Speech. Lang. Hear Res. 28 (1985) 265-272.

[18] D. H. Keefe, E. Levi, Maturation of the middle and external ears: acoustic power-based responses and reflectance tympanometry, Ear Hear. (17) 1996 361-373.

[19] J. C. Saunders, J. A. Kaltenback, E. M. Relkin, The structural and functional development of the outer and middle ear. In: R. Romand, M. R. Romand (Eds), Development of Auditory and Vestibular systems, Academic Press, New York, 1983, pp. 3-25.

[20] B. J. Anson, J. A. Donaldson, Surgical Anatomy of the Temporal Bone and Ear, 3rd ed, W. B. Saunders, Philadelphia, 1981.

[21] A. Ikui, I. Sando, S. Haginomori, M. Sudo, Postnatal development of the tympanic cavity: A computer-aided reconstruction and measurement study, Acta. Otolaryngol. 120 (2000) 375-379.

[22] L. Qi, H. Liu, J. Lutfy, W. R. Funnell, S. J. Daniel, A nonlinear finite-element model of the newborn middle ear, J. Acoust, Soc. Am.120 (2006) 3789-3798.
[23] L. Holte, R. M. Cavanaugh, R. H. Margolis, Ear canal wall mobility and tympanometric shape in young infants. J. Pediatr. 117 (1990) 77-80.
[24] C. A. Sanford, M. P. Feeney, Effects of maturation on tympanometric wideband acoustic transfer functions in human infants, J. Acoust. Soc. Am. 124 (2008) 2106-2122.
[25] V. Aithal, J. Kei, C. Driscoll, A. Swanston, K. Roberts, M. Murakoshi, H. Wada, Normative sweep frequency impedance measures in healthy neonates, J. Am. Acad. Audiol, 25 (2014) 343-354.
[26] M. Murakoshi, N. Yoshida, M. Sugaya, Y. Ogawa, S. Hamanishi, H. Kiyokawa, R. Kakuta, M. Yamada, R.Takahashi, S.Tanigawara, S. Matsutani, T. Kobayashi, H. Wada , Dynamic characteristics of the middle ear in neonates, Int. J. Pediatr. Otorhinolaryngol. 77 (2013) 504-512.
[27] JCIH, Joint committee on infant hearing, Position statement: Principles and guidelines for early hearing detection and intervention programs, Pediatrics 120 (2007) 898-921.
[28] S. Aithal, V. Aithal, J. Kei, C. Driscoll, Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia, J. Am. Acad. Audiol, 23 (2012) 1-13.
[29] C. Driscoll, J. Kei, B. McPherson, Transient evoked otoacoustic emissins in six year old school children: A normative study, Scand. Audiol. 29 (2000) 103-110.
[30] J. Kei, J. Allison-Levick, J. Dockray, R. Harrys, C. Kirkegard, et al. High-frequency (1000 Hz) tympanometry in normal neonates, J. Am. Acad. Audiol. 14 (2003) 20-28.
[31] R. Mazlan, High frequency (1000 Hz) tympanometry and acoustic reflex findings in newborn and 6-weeks-old infants, PhD Thesis, University of Queensland, Brisbane, 2009.
[32] H. Wada, T. Yokobori, T. Kakizaki, T. Kobayashi, T. Takasaka, Mechanical property of ossicles and prosthesis, Otol. Jpn. (6) 1996 42-46.
[33] L. Qi, W. R. Funnell, S. J. Daniel, 2008. A nonlinear finite-element model of the newborn middle ear, J. Acoust. Soc. Am. 124 (2008) 337-347.
[34] J. Kei, R. Mazlan, High-Frequency (1000 Hz) Tympanometry: Clinical applications. In: J. Kei, F. Zhao, (Eds). Assessing middle ear function in infans, Plural, San Diego, 2012, pp.39-67.
[35] N. Shahnaz, T. Miranda, L. Polka, Multifrequency tympanometry in neonatal intensive care unit and well babies, J. Am. Acad. Audiol. 19 (2008) 392-418.

[36] C. A. Sanford, D. H. Keefe, Y-W. Liu, D. F. Fitzpatrick, R. W. McCreery, D. E. Lewis, M. P. Gorga, Sound-conduction effects on Distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry, Ear Hear. 30 (2009) 635-652.

[37] S. Aithal, J. Kei, C. Driscoll, A. Khan, Normative wideband reflectance measures in healthy neonates, Int. J. Pediatr. Otorhinolaryngol. 77 (2013) 29-35.

Chapter 4: Sweep Frequency Impedance measures in young infants: Developmental characteristics from birth to 6 months

4.1 Background

The outer and middle ear undergoes rapid developmental changes during the first few months of life. These developmental changes in early infancy could have a significant effect on the dynamic behaviour of the outer and middle ear. Hence, there is a need to track developmental changes of the outer and middle ear at various ages from birth to 6 month using SFI measures.

The study of the effect of age on SFI measures is presented in Chapter 4 of this thesis. This chapter is based on the manuscript published in the *International Journal of Audiology.* This paper is inserted into this thesis with minor modifications. Only the formatting of the section sub-headings and numbering of tables and figures have been modified from the original manuscript to match the thesis format. The referencing format of the paper is maintained as per the journal format.

Aithal, V., Kei, J., Driscoll, C., Murakoshi, M., & Wada, H. (2016). Sweep Frequency Impedance measures in young infants: Developmental characteristics from birth to 6 months. Manuscript accepted for publication in *International Journal of Audiology.* DOI:10.1080/14992027.2016.1244867

4.2 Abstract

Objective: To study the developmental characteristics of sweep frequency impedance (SFI) measures in healthy infants from birth to 6 months.

Design: All infants were assessed using high frequency tympanometry (HFT), distortion product otoacoustic emission (DPOAE) and SFI tests. SFI measures consisted of measurement of resonance frequency (RF) and mobility (ΔSPL) of the outer and middle ear.

A mixed model analysis of variance was applied to the SFI data to examine the effect of age on RF and ΔSPL.

Study Sample: Study included 117 ears from 83 infants of different age groups from birth to 6 months.

Results: The mean RF of the outer ear increased from 279 Hz at birth to 545 Hz at 4 months, whereas mean ΔSPL of the outer ear decreased from 7.9 dB at birth to 3.7 dB at 4 months of age. In contrast, the mean RF and ΔSPL of the middle ear did not change significantly with age up to 6 months.

Conclusions: Developmental characteristics should be considered when evaluating the function of the outer and middle ear of young infants (≤ 6 months) using the SFI. The preliminary normative SFI data established in this study may be used to assist with the evaluation.

Key words: Electrophysiology, Middle ear, Paediatric, Instrumentation, Sweep Frequency Impedance

Abbreviations:

AABR	Automated Auditory Brainstem Response
ABR	Auditory Brainstem Response
ANOVA	Analysis of Variance
DPOAE	Distortion Product OtoacousticEmission
HFT	High Frequency Tympanometry
OAEs	Otoacoustic Emissions
Ps	Static Pressure
RF	Resonance Frequency
SFI	Sweep Frequency Impedance
SPL	Sound Pressure Level

4.3 Introduction

Measures of auditory function such as pure tone audiometry, tympanometry, otoacoustic emissions (OAEs) and auditory brainstem response (ABR) are affected by the condition of the outer and middle ear. Pathological conditions such as obstruction of outer ear or middle ear disorder may result in abnormal test findings. However, non-pathological factors may also result in significant variations in test findings. These factors may include

the size of the ear canal and middle ear cavity, elastic properties of the ear canal wall, orientation and thickness of the tympanic membrane and stiffness of the middle ear. From a clinical perspective, it is important to distinguish between changes in test findings attributable to pathological conditions and those attributable to non-pathological conditions such as maturation.

One common non-pathological condition that influences results of some acoustic measures is the maturation of the auditory system. The outer and middle ear system undergoes fast developmental changes from birth to six months of age. The external ear canal is straighter and approximately 50% shorter in length in young infants (< 6 months) than in adults (Saunders et al., 1983; Keefe et al., 1993). The infant ear canal wall has no bony portion (Anson & Donaldson, 1981), and is completely surrounded by a thin layer of elastic cartilage at birth , making it highly compliant, flaccid and prone to collapse (Sprague et al., 1985; Holte et al., 1990; Keefe et al., 1993). The diameter and length of the ear canal increase from birth to 24 months of age (Saunders et al., 1983; Keefe et al., 1993).

The tympanic membrane is nearly horizontal relative to the external auditory canal axis in neonates, whereas it is approximately 45 degrees in adults (Anson & Donaldson, 1981; Qi et al., 2006). The tympanic membrane in neonates is thicker than that in adults. The pars tensa region varies in thickness from 0.1 to 1.5 mm in neonates (Ruah et al., 1991), whereas it ranges from 0.04 to 0.12 mm in adults (Kuypers et al., 2006).

The volume of the middle ear cavity in neonates, which includes the tympanic cavity, aditus and antrum, mastoid antrum and mastoid air cells, is small and increases postnatally until late teenage years. The volume of the tympanic cavity is reported to be approximately 330 mm^3 in neonates (Qi et al., 2008), 452 mm^3 in 3-month-old infants and 640 mm^3 in adults (Ikui et al., 2000).

The middle ear of a newborn is not completely aerated. It contains amniotic fluid, exudates, mesenchyme, mucoid effusions and other materials (Palva et al., 1999). Aeration usually occurs during the first 48 hours, but fluid and other materials are reported to stay for a prolonged period of time in some ears. Approximately 50% of ears retain middle ear fluid by the end of the first 24 hours after birth, decreasing to 27% after 48 hours, and 13% after 2 weeks (Roberts et al., 1992).

The middle ear of neonates is dominated by mass and resistance. As the child grows, the electro-mechanical properties of the outer and middle ear system are altered with increasing stiffness during the infancy period (Holte et al., 1991). The low stiffness of the ear canal walls in neonates suggests that the external ear canal wall, being elastic, could vibrate in response to sound stimulation. For instance, on pressurization as in tympanometry, the diameter of ear canal increased by an average of 18.3% under positive pressure or decreased by an average of 28.2% under negative pressure of its original value (Holte et al., 1990).

As expected, these developmental changes are likely to affect the results of middle ear measures, including single-frequency and multi-frequency tympanometry (MFT), wideband acoustic immittance (WAI) and sweep frequency impedance (SFI) measures. Single low frequency (226 Hz) tympanometry reveals lower static admittance, broader tympanic width and the appearance of notches (Hunter & Blankenship, 2017) . Single low frequency tympanometry in infants younger than 6 months has also been shown to produce normal tympanograms in ears with middle ear fluid (Hunter & Margolis, 1992; Baldwin, 2006) and abnormal results in ears with normal middle ear function (Keefe & Levi, 1996; Rhodes et al., 1999). The MFT procedure uses either a sweep frequency technique at multiple applied air pressures to the ear canal or a sweep pressure technique using tone of multiple discreet frequencies (Margolis et al., 1985; Margolis & Goycoolea, 1993). While measurements of complex admittance, such as susceptance and conductance and resonance frequency (RF) of the middle ear, are useful (Hunter & Margolis, 1997; Hunter et al., 2010), they produce unusual tympanometric patterns in newborns which are difficult to classify (Calandruccio et al., 2006). Although normative aspects of wideband absorbance (WBA) are well researched in newborns (Keefe et al., 2000; Feeney & Sanford, 2008; Sanford et al., 2009; Hunter et al., 2010; Werner et al., 2010; Aithal et al., 2013; Hunter et al., 2015), there are limited data on the developmental aspects of WBA (Keefe et al., 1993; Sanford & Feeney, 2008; Kei et al., 2013; Aithal et al., 2014) and no data on the resonance frequency of the outer ear of young infants.

At present, new MFT techniques that measure acoustic-mechanical properties over a wide frequency range have been developed to assess the outer and middle ear function. Two such techniques are SFI (Murakoshi et al., 2012; Zhao & Wang, 2012) and WAI measures (Keefe et al., 2000; Murakoshi et al., 2012; Kei et al., 2013). WAI is an emerging tool to

assess outer and middle ear function using clicks. WAI measures include reflectance, admittance and phase analysed across a broad frequency range (200 to 8000 Hz) (Rosowski et al., 2013; Keefe et al., 2015). Wideband reflectance (WBR) is a potentially useful technique to measure the acoustic-mechanical properties of outer and middle ear because it is relatively free of ear canal effects that complicate admittance measurements at high frequencies above 2000 Hz (Hunter & Margolis, 1997; Rosowski et al., 2013).

At present, measures such as high frequency tympanometry (HFT) using a 1000 Hz probe tone (Purdy & Williams, 2002; Kei et al., 2003; Margolis et al., 2003) and WAI measures (Keefe et al., 1993; Shahnaz, 2008; Keefe, 2008 ; Aithal et al., 2014) are recommended as tests of middle ear function in neonates and young infants. Although normative HFT data are available in neonates (Kei et al., 2003; Margolis et al., 2003; Calandruccio et al., 2006), there are still unresolved issues regarding their measurement and interpretation (Kei & Zhao, 2012). In addition, studies that have compared the test performance of HFT and WBR with distortion product otoacoustic emissions (DPOAEs) have found that WBR predicted DPOAE outcomes better than HFT measures (Sanford et al., 2009; Hunter et al., 2010).

The SFI, developed in the 1990s, measures the resonance frequency (RF) and mobility (Δ SPL) of the outer and middle ear system at different static pressures in the ear canal as well as tympanometric peak pressure (TPP, the pressure at which the SPL attains maximum) (Wada & Kobayashi, 1990; Murakoshi et al., 2012; Zhao & Wang, 2012). Although the technology "sweep frequency impedance, SFI" appears to measure impedance, it actually measures the sound pressure in the ear canal while a sweeping tone is presented under various static pressure levels in the ear canal. From the SFI measures, the dynamic behaviour of the outer and middle ear can be described in a graph showing the sound pressure level (in dB SPL) against frequencies from 100 to 2200 Hz at various static pressures applied to the ear canal. From the SPL results, the RF and Δ SPL can be measured (Wada et al., 1989). The SFI is faster and more accurate than MFT (Murakoshi et al., 2012). The dynamic behaviour of the outer and middle ear system, as measured using SFI meter, of a normally hearing adult is different from that of a healthy newborn. Figure 4.1(a) shows the typical SFI result obtained from a normal healthy neonate at ambient pressure which shows two variations in sound pressure, one around RF1(low resonance frequency) and the other around RF2(high resonance frequency). On the other hand, the SFI result at ambient pressure for a

healthy adult reveals only one variation at RF2 (Aithal et al., 2016) (Figure 4.1(b)). In a broader sense, although both SFI and WAI utilise a wider frequency range for measurements, they assess different aspects of middle ear function. WAI assesses wideband acoustic transfer functions of the middle ear over a wide frequency range from 250 to 8000 Hz (Keefe et al., 1993). On the other hand, SFI does not measure the admittance of the middle ear system. It measures the dynamic behaviour of the outer and middle ear system by investigating how the RF and Δ SPL change when the ear canal static pressure changes (Wada et al., 1989; Murakoshi et al., 2012). When the ear canal pressure is greater than the TPP (or ambient pressure), RF increases and Δ SPL decreases, indicating greater stiffness of the outer and middle ear. When the ear canal pressure is smaller than the TPP (or ambient pressure), RF increases and Δ SPL decreases at a much faster rate until the ear canal collapses under significant negative ear canal pressure.

Sound energy is transmitted most efficiently to the middle ear at a frequency corresponding to the resonance of the middle ear because the ear drum vibrates with the largest displacement amplitude at that frequency. Using a finite-element method, it has been shown that the maximum value of the middle ear transmission gain (i.e. forward and backward transmission) is obtained at the RF. The peak value of the gain depends on the mobility of the middle ear. Such middle ear dynamic characteristics can be easily measured in terms of RF and the mobility of the middle ear (Δ SPL) using a SFI meter (Zhao et al., 2003). It was suggested that the dynamic characteristics of the outer and middle ear can provide insight into pathological conditions in patients with conductive disorders. Whereas the SFI has been reported to be useful in the diagnosis of various middle ear pathologies in children and adults, its application to evaluating the outer and middle ear characteristics in neonates is only just emerging (Wada et al., 1989; Wada & Kobayashi, 1990; Murakoshi et al., 2012).

The SFI technology has been successfully trialled in infants by Murakoshi et al. (2013), Aithal et al. (2014) and Aithal et al. (2016). These studies have shown that the SFI can be used to measure the RF and ΔSPL in neonates. The resonance that occurred in the low-frequency region (e.g., 210 – 420 Hz) was considered to be associated with the movement of the elastic external ear canal wall (Murakoshi et al., 2013; Aithal et al., 2014; Hamanishi et al., 2015), while the resonance that occurred in the higher frequency region (e.g., 830 -1500 Hz) was considered to be associated with the movement of the middle ear

components (Wada et al., 1989; Murakoshi et al., 2012). Recent reports have shown that absence of resonance in higher frequency region (RF2) with the presence of resonance in the low-frequency region (RF1) in newborns is clinically significant as it can potentially identify ears with middle ear pathology (Murakoshi et al., 2012; Aithal et al., 2016). Given such promising SFI results as an assessment of middle ear pathology, the authors suggested that the SFI technology may be used with neonates for evaluating outer and middle ear function (Murakoshi et al., 2013; Aithal et al., 2014; Aithal et al., 2015; Aithal et al., 2016).

In view of the rapid developmental changes in the auditory system during the first 6 months of life, it is expected that the SFI findings also change as a function of age. Therefore, when the SFI is used as a diagnostic tool to identify conductive conditions in infants, age-appropriate normative SFI data will be required. The aim of the present study was to investigate the developmental characteristics of the SFI measures in infants from birth to 6 months with a view to establish preliminary normative SFI data for each age group.

4.4 Methods

4.4.1 Subjects and test environment

This study was approved by the Human Research Ethical Committee of The Townsville Hospital and Health Service, and the University of Queensland Behavioural and Social Science Ethical Review Committee (Appendix 1). Parents of healthy newborns in the maternity ward of The Townsville Hospital were informed of the study by nurses. Parents provided written consent for their children to be included in the study (Appendix 2).

All infants were born at full term with normal birth weight and no medical complications or risk factors for hearing loss. They were tested at birth with follow-up appointments scheduled at 1, 2, 4 and 6 months of age. When an infant attended more than one follow-up appointment, data collected at only one of the appointments were included in the analysis. Hence, this study only included cross-sectional data of infants at various time intervals. The number of infants enrolled included 24 newborns (0 month), 16 infants at 1 month, 13 infants at 2 months, 17 infants at 4 months, and 13 infants at 6 months of age. Overall, the study included 117 ears from 83 infants (47 males and 36 females). All infants were Caucasians. Details of infants included in the study are shown in Table 4.1.

Table 4.1. Details of infants included in the study who passed HFT and DPOAE

	N (subjects)				N (ears)		
Age group	Age range	Male	Female	Total	Right	Left	Total
0 m	21 - 70 h	9	15	24	17	13	30
1 m	29 - 40 days	8	8	16	14	14	28
2 m	55 - 60 days	9	4	13	6	12	18
4 m	115-135 days	13	4	17	14	8	22
6 m	170 - 190 days	8	5	13	9	10	19
Total		47	36	83	60	57	117

Initial testing with the newborns was performed in a quiet room in the maternity unit. The ambient noise level in the room was less than 40 dBA. Only neonates who passed the State mandated automated auditory brainstem response (AABR) screening test as part of the universal newborn hearing screening program were recruited for the study. The initial AABR screening was performed by a trained nursing staff using an ALGO3 device (Natus Medical Inc.) with clicks presented at 35 dB nHL. Passing the AABR screen was necessary to ensure the likelihood of normal auditory function.

The HFT, DPOAE and SFI tests were administered by a clinical audiologist following the AABR screen. Wherever possible, the HFT, DPOAE and SFI tests were completed for both ears of each newborn with no particular test order. The most accessible ear was tested first. All tests were completed on one ear before the second ear was attempted. The second ear was tested if the infant was well settled and there was adequate time for testing. The age of testing varied from 21 to 70 hours at birth, 29 to 40 days at 1 month, 55 to 60 days at 2 months, 115 to 135 days at 4 months and 170 to 190 days at 6 months.

4.4.2 Procedure

Evaluations with the infants aged 1 to 6 months were performed in a sound treated room at the Audiology department. The ambient noise levels in the sound booths were less than 35 dBA. Infants were seen after feeding while in natural sleep or in an awake but quiet state. For infants in each age group, only the ears that passed a test battery, consisting of HFT with 1000 Hz probe tone and DPOAE test, were included in the study. The present study used a test battery approach (pass in DPOAE and HFT) as the reference standard for normal outer/middle ear function. Although a pass in DPOAE or HFT does not necessarily rule out

outer/middle ear dysfunction (Driscoll et al., 2000; Aithal et al., 2012), a pass in both tests constituted a more stringent "reference standard" than a single-test reference standard to ensure an unobstructed conductive pathway in infants.

The HFT was performed using a Madsen Otoflex 100 acoustic immittance device (GN Otometrics) with a 1000-Hz probe tone. Admittance (Ya) was measured as the pressure was swept from +200 to -400 daPa at a rate of 400 daPa/sec. Pass criteria were a single positively peaked tympanogram with the middle ear pressure between 50 and -150 daPa and peak compensated static admittance Ypc (+200 daPa tail to peak) of at least 0.2 mmho (Mazlan et al., 2009).

The DPOAE test was performed using a Biologic Navigator Plus device. DPOAEs were obtained in response to stimulation by pairs of primary tones. The f2/f1 frequency ratio was 1.2 for each primary pair. The level of f1 was 65 dB SPL and f2 was 55 dB SPL. The 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).

The SFI test was performed using a new SFI unit developed for testing neonates (Murakoshi et al., 2013). The SFI unit and its calibration have been described in detail elsewhere (Murakoshi et al., 2013; Aithal et al., 2014). The SFI unit consisted of a personal computer, an AD/DA converter, a probe system, a stepping motor, an air pump, a pressure sensor, and a pressure relief valve. The probe consisted of 3 tubes: the first tube to apply static pressure (Ps) to the ear canal, the second tube to deliver sound to the external ear canal via an earphone, and the third tube to measure sound pressure in the external ear canal using a microphone. A specially designed cuff suitable for testing neonates was attached to the tip of the probe to obtain a hermetic seal during testing. This new SFI unit was controlled using LabView under MS WINDOWS.

The SFI unit was programmed to perform the test procedure. During the SFI test, the sound pressure level in the ear canal was measured as the frequency of the pure tone stimulus was swept from 100 to 2200 Hz while the external auditory canal static pressure (Ps) was held constant at +200 daPa. This measurement was repeated with Ps reduced in 50 daPa steps down to -200 daPa. The entire SFI procedure was automated and it took 40 seconds to

complete the test in each ear. The sweeping probe tone level was kept below 75 dB SPL to reduce the risk of eliciting acoustic stapedial reflexes. While the SFI results measured at multiple static pressures provide a comprehensive view of the acoustic-mechanical properties of the outer and middle ear, Aithal et al. (2015) found that measurements made at ambient pressure (0 daPa) can provide useful clinical information about the status of the outer and middle ear. For the purpose of the present study, measurements performed at both ambient pressure and TPP were included in the analyses for comparison purposes.

Previous studies have demonstrated that the SPL curves obtained from neonates have shown two variations i.e., low frequency (210-420 Hz) and high frequency (830-1500 Hz) regions (Murakoshi et al., 2013; Aithal et al., 2014). This suggests that there are two vibrating elements in the neonatal auditory system, possibly due to external and middle ear components. The second variation in the higher frequency region (RF2) was similar to that of adult middle ear resonance frequency reported in a previous study (1170 ± 270 kHz, $n = 275$) (Wada et al., 1998).

The first variation in the low-frequency region (RF1) was thought to be caused by an element other than the middle ear. It was considered to be associated with the movement of the external ear canal wall as Young's modulus of which is estimated to be 0.36 times as much as that of adults, i.e., 36-364 kPa (Saunders et al., 1983; Qi et al., 2006). It was also reported that the resonance movements of the neonatal ear canal to be lower than 450 Hz based on the ear canal impedance measurement (Keefe et al., 1993). In addition, studies based on a neonatal external ear canal physical model using agarose gel and a numerical model using finite element method suggest that the external ear canal wall exhibits intrinsic oscillatory behaviour at around 300 Hz (Murakoshi et al., 2013; Hamanishi et al., 2015). Hence the first variation of the SPL curve obtained from neonates in the low-frequency region (RF1) was considered to be related to the resonance of the neonatal external ear canal wall movements

4.5 Results

In this study, the resonance frequency of the outer ear (RF1) and middle ear (RF2), and mobility of outer ear (ΔSPL1) and middle ear (ΔSPL2) were examined. The resonance frequency (RF) and mobility (ΔSPL) of the ear canal and middle ear are measured directly

from the sound pressure curves obtained at ambient pressure and TPP in the frequency region around resonance. The sound pressure level difference (ΔSPL) between the bottom of the curve and its peak reflects the magnitude of the mobility at the resonance frequency (Wada et al., 1993).

Figure 4.1(a) shows the typical SFI result at ambient pressure (0 daPa) obtained from a one-day-old neonate who passed the test battery of HFT and DPOAE tests. The SPL curve at ambient pressure/TPP shows two variations in sound pressure (RF1 and RF2) whereas an adult healthy ear shows only one inflexion (RF2) (Figure 4.1(b)). In neonates, the sound pressure level (SPL) curve shows an increase in SPL at two frequency regions, Fb1 to Fa1 and Fb2 to Fa2 with corresponding SPL at Pb1, Pa1, Pb2 and Pa2, respectively. Previous studies have shown that the greatest variation (displacement) of SPL (ΔSPL) occurs at median frequencies RF1 and RF2, which are halfway between the frequencies Fa1 and Fb1, and between Fa2 and Fb2, respectively (Wada et al., 1989; Murakoshi et al., 2013; Aithal et al., 2014). Murakoshi et al (2013) and Aithal et al (2014) identified the first resonance at RF1 as the frequency at which the SPL varies considerably between Fb1 to Fa1. Hence, RF1 and the corresponding variation in SPL (Δ SPL1) are defined as shown in Equations (4.1) and (4.2) (Murakoshi et al., 2012; Murakoshi et al., 2013).

First Resonance Frequency, RF1 = (Fa1+Fb1) / 2 Equation (4.1)

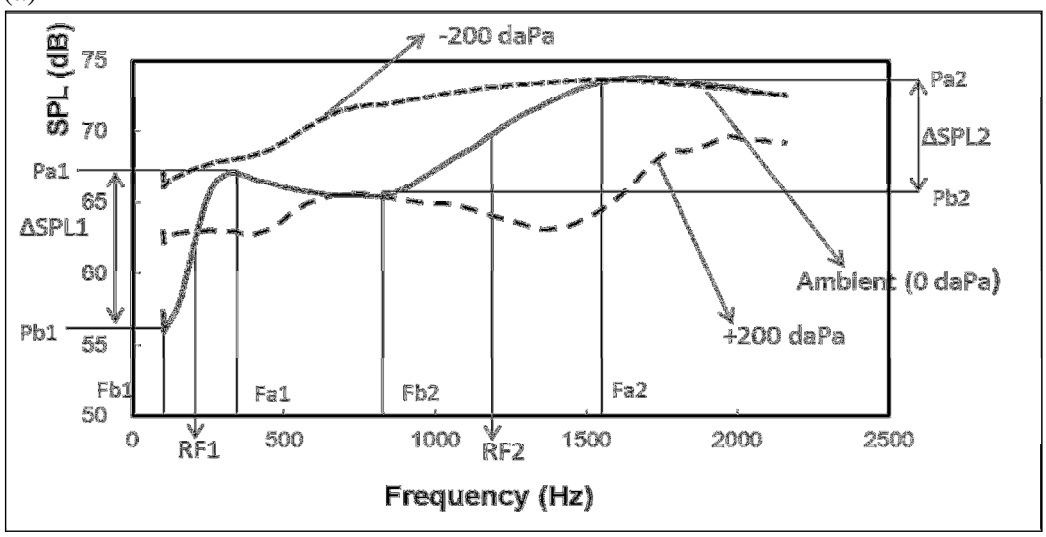
Sound pressure change at RF1, ΔSPL1 = Pa1 - Pb1 Equation (4.2)

Similarly, the second resonance frequency (RF2) and the corresponding variation in SPL (Δ SPL2) are defined as shown in Equations (4.3) and (4.4).

Second Resonance Frequency, RF2 = (Fa2+Fb2) / 2 Equation (4.3)

Sound pressure change at RF2, ΔSPL2 = Pa2 - Pb2 Equation (4.4)

(a)



(b)

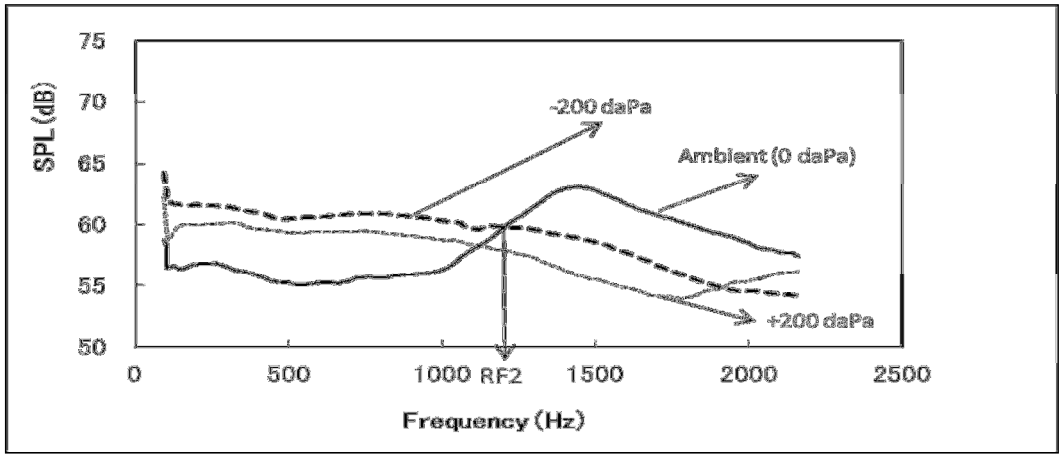


Figure 4.1. Typical SFI results obtained (a) from a healthy one-day-old neonate who passed the test battery. The SPL curve at ambient pressure shows two variations in sound pressure (RF1 and RF2). The static ear canal pressure (daPa) applied were +200, 0 (ambient), and -200 daPa. Pa1 and Pb1 are the maximum and minimum sound pressures, and Fa1 and Fb1 are the frequencies corresponding to these sound pressures (first variation, RF1). Pa2 and Pb2 are the maximum and minimum sound pressures, and Fa2 and Fb2 are the frequencies corresponding to these sound pressures (second variation, RF2). RF1 and RF2 are defined by (Fa1+Fb1)/2 and (Fa2+Fb2)/2, respectively. ΔSPL1 and ΔSPL2 are defined by (Pa1- Pb1) and (Pa2 - Pb2), respectively; (b) from a normal hearing adult who passed 226 Hz tympanometry. Note: The SFI curve at ambient pressure shows only one inflexion (RF2). RF = resonance frequency.

As shown in Figure 4.1(a), when static pressure (Ps) = 0 daPa (ambient pressure), RF1=220 Hz and RF2 =1200 Hz. The corresponding SPL variations, Δ SPL1 = 10 dB and ΔSPL2 = 7 dB. These results indicate that there are two distinct resonance frequencies at which the SPL varies considerably.

Table 4.2 shows a summary of descriptive statistics of the SFI data, showing the number of ears, mean, standard deviation (SD) and median for RF1, RF2, ΔSPL1 and ΔSPL2 for infants aged 0-, 1-, 2-, 4- and 6-months. The table also shows the SFI data obtained at ambient pressure and TPP. Results of t-tests applied to ambient and TPP SFI data showed significant differences for RF1 and ΔSPL1 at birth and ΔSPL1 at 1month. The mean resonance frequency (RF1) increased from 279 Hz (SD = 47 Hz) at birth (0-month) to 545 Hz (SD = 134 Hz) at 4-months of age, whereas mean mobility of the ear canal (ΔSPL1) decreased from 7.9 dB (SD = 2.7 dB) at birth (0- month) to 3.7 dB (SD = 2.5 dB) at 4-months of age. RF1 and ΔSPL1 could not be measured in 6 ears of 4-month-old and all ears of 6-month-old infants because their SPL curves did not show the first variation corresponding to the resonance of the outer ear. Nevertheless, their SPL curves still showed the second variation corresponding to the resonance of the middle ear where RF2 and ΔSPL2 were measured. The mean change in RF2 ranged from 1174 Hz to 1395 Hz and mean change in ΔSPL2 ranged from 3.8 dB to 5.0 dB across the 6-month period with no clear trend or patterns observed.

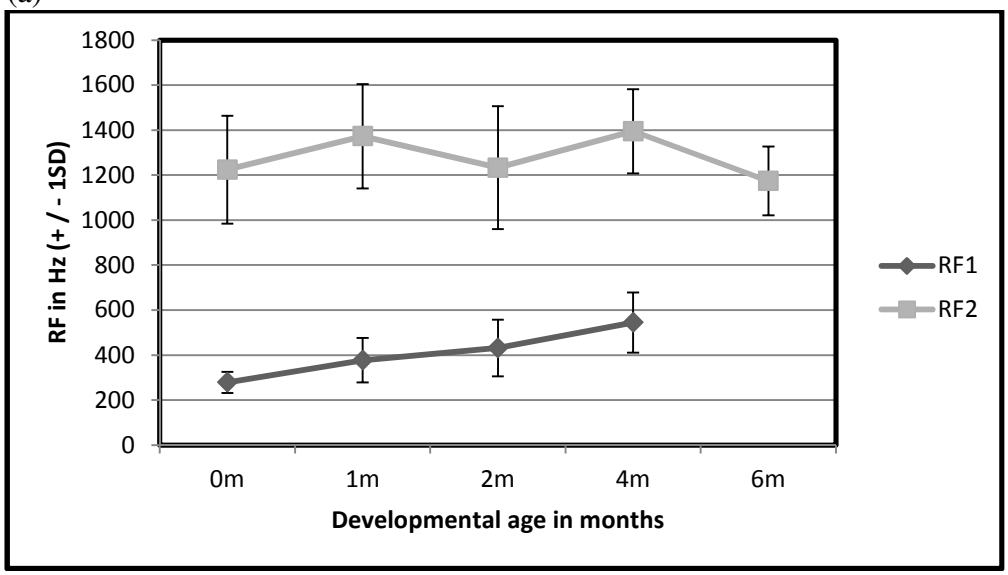
Table 4.2. Mean, SD, and percentile SFI data (RF1, RF2, ΔSPL1 and ΔSPL2) for infants aged 0-, 1-, 2-, 4- and 6 months.

Age Group		**RF1 (Hz)**		**SPL1 (dB)**		**RF2 (Hz)**		**SPL2 (dB)**	
		Ambient	**TPP**	**Ambient**	**TPP**	**Ambient**	**TPP**	**Ambient**	**TPP**
	N (ears)	30	30	30	30	30	30	30	30
	Mean	279	307	7.9	7	1224	1239	5	5
	SD	47	73	2.7	2.3	240	226	2	2
0 m	5%le	200	211	2.9	1.8	870	920	1.5	1.5
	50%le	275	276	8.1	6.8	1220	1235	5.1	4.6
	95%le	370	462	12.6	11.3	1692	1685	8.1	8.4
t		-3.66		2.26		-0.92		0.27	
df		29		29		29		29	
p		0.001*		0.03*		0.37		0.8	
	N (ears)	28	28	28	28	28	28	28	28
	Mean	377	389	5.6	4.5	1373	1338	3.8	4
	SD	99	105	2	2	232	197	2.5	2.4
1 m	5%le	250	254	2.4	1.2	1044	1064	0.6	0.7
	50%le	355	387	5.5	4.2	1375	1320	3.5	3.4
	95%le	556	585	9.6	8.2	1923	1783	9.1	9.3
t		-1.14		4.45		2.04		-0.66	
df		27		27		27		27	
p		0.27		0.00*		0.051		0.52	
	N (ears)	18	18	18	18	18	18	18	18
	Mean	432	452	4.2	3.9	1233	1240	4.1	4.4
	SD	126	152	2.6	2.4	273	247	2.8	2.8
2 m	5%le	260	251	1	1.5	910	910	0.3	0.4
	50%le	419	450	4	3.3	1174	1174	3.9	3.7
	95%le	680	600	5.6	5.8	1708	1619	8.1	9.4
t		-1.4		0.79		-0.25		-1.7	
df		17		17		17		17	
p		0.18		0.44		0.81		0.11	
	N (ears)	16	16	16	16	22	22	22	22
	Mean	545	546	3.7	3.5	1395	1397	4.1	4
	SD	134	131	2.5	2.1	187	176	3.6	3.2
4 m	5%le	250	270	1.4	1.3	1131	1042	0.4	0.4
	50%le	594	585	2.5	2.8	1375	1374	2.7	3.1
	95%le	650	680	7.8	6.8	1747	1737	10.8	11
t		-0.24		0.64		-0.12		0.15	
df		15		15		21		21	
p		0.81		0.53		0.91		0.88	
	N (ears)	19	19	19		19	19	19	19
	Mean	NA	NA	NA	NA	1174	1139	4.7	4.5
	SD	NA	NA	NA	NA	153	135	2.8	2.5
6 m	5%le	NA	NA	NA	NA	800	780	1.3	1.7
	50%le	NA	NA	NA	NA	1170	1160	4	3.8
	95%le	NA	NA	NA	NA	1479	1359	9.5	8.5
t						0.92		0.28	
df						18		18	
p						0.37		0.78	
Note: *significant with p<0.05. NA means results not available									

Results of t-tests applied to ambient and TPP SFI data are shown.

Figure 4.2(a) illustrates the trend of mean SFI measures for resonance frequencies (RF1 and RF2) and Figure 4.2(b) shows the trend of mean mobility (ΔSPL1 and ΔSPL2) for infants aged 0- (birth), 1-, 2-, 4- and 6- months. An increasing trend for mean RF1 and a decreasing trend for mean ΔSPL1 with age were observed. In contrast, mean RF2 and Δ SPL2 remained relatively stable with no observable trend.

(a)



(b)

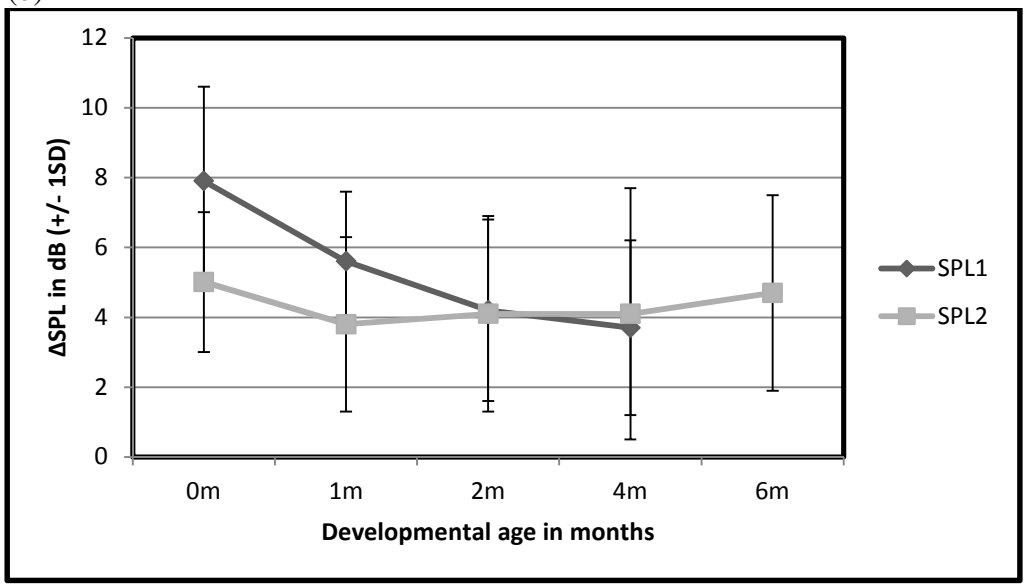


Figure 4.2. Mean SFI measures in infants (0 mo of age) and 1-, 2-, 4- and 6-mo-old infants. (a) Graph showing mean ± 1SD for RF1 and RF2. (b) Graph showing mean ± 1SD for Δ SPL1 and Δ SPL2

To investigate the effect of age on RF and ΔSPL, a general linear model univariate analysis of variance (ANOVA) was applied separately to the RF1, RF2, ΔSPL1 and ΔSPL2 data (dependent variable) with age as an independent (fixed) factor. Table 4.3 shows the ANOVA results for SFI measures across the age groups, indicating significant main effects for RF1, RF2 and ΔSPL1, but not for ΔSPL2. The main effects for RF2 were significant, but not systematic. The magnitudes of the effects, as shown by partial eta squared values, were RF1 (0.48), ΔSPL1 (0.31), RF2 (0.14), and ΔSPL2 (0.03).

Table 4.3. ANOVA results of SFI data across age groups.

	F value	df	p value	Partial eta squared value	Observed power
RF1	26.5	3, 88	0.00*	0.48	1
RF2	4.43	4, 112	0.00*	0.14	0.93
Δ SPL1	13.42	3, 88	0.00*	0.31	1
Δ SPL2	0.89	4, 112	0.47	0.03	0.28
*indication of statistical significance. $p<0.005$					

To further investigate the effect of age on RF and ΔSPL, post hoc multiple pair-wise comparison tests with Bonferroni correction factor were performed on SFI data. Table 4.4 shows the results of post hoc analysis across different age groups. In general, RF1 and ΔSPL1 of the 0-month group were significantly different from those of the 1-, 2- and 4-month groups. There was also significant difference in RF1 between the 1- month and 4- month groups as well as between the 2-month and 4-month groups. However, RF2 did not change systematically with age, but there were significant differences in RF2 between the 1-month and 6-month groups as well as between the 4-month and 6-month groups. Not surprisingly, ΔSPL2 did not show any significant variations across all age groups.

Table 4.4. Results of post hoc multiple pair-wise comparisons with Bonferroni adjustments for SFI measures between age groups

Group	0 m vs 1 m	0 m vs 2 m	0 m vs 4 m	0 m vs 6 m	1 m vs 2 m	1 m vs 4 m	1 m vs 6 m	2 m vs 4 m	2 m vs 6 m	4 m vs 6 m
RF1	0.00*	0.00*	0.00*	-	0.43	0.00*	-	0.01*	-	-
RF2	0.12	1.00	0.07	1.00	0.39	1.00	0.03*	0.24	1.00	0.02*
Δ SPL1	0.00*	0.00*	0.00*	-	0.42	0.13	-	1.00	-	-
Δ SPL2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*indication of statistical significance. p<0.05										

4.6 Discussion

The present study developed preliminary normative data for the resonance frequency and mobility of the outer/middle ear system in newborns and young infants using a cross sectional design. These SFI data showed that variations in resonance frequency of the outer ear and middle ear are quite different in the first six months of life which could be used as a developmental marker, especially for ear canal development. There was clear evidence of maturation of the outer ear but there was no clear evidence of maturation of the middle ear over this period as revealed by the SFI results (Table 4.2). Although significant differences were noted only between ambient and TPP for RF1 and ΔSPL1 at birth and ΔSPL1 at 1 month, SFI data obtained at ambient pressure were used for analysis in the current study as no significant differences were noted for other age groups. No significant differences were observed between ambient and TPP for RF2 and ΔSPL2 from birth (0m) to 6 months.

The present SFI data showed that the resonance frequency of the outer ear (RF1) increased significantly with age from 0 to 4 months. Previous studies have noted that the resonance frequency of an infant ear was lower than 550 Hz (Weatherby & Bennett, 1980; Holte et al., 1991; Meyer et al., 1997). For example, Meyer et al. (1997) measured the resonance frequency of an infant's ear from 2 weeks to 6 and half months and noted that it remained below 550 Hz until the resonance disappeared at 3.5 months of age. Similarly using

multi-frequency tympanometry, Holte et al. (1991) observed that the frequency of the first resonance in an infant's ear was at about 450 Hz and that the resonance disappeared by 4 months of age. In general, the results of these studies are consistent with that of the present study whereby the resonance frequency of the outer ear (RF1) was measurable up to 4 months of age and it was below 550 Hz at 4 months of age. However, direct comparison of resonance frequency results of young infants with other studies is not possible because of the lack of research data for young infants in the literature.

The present study supports the view that low frequency resonance recorded by Meyer et al. (1997) and Holte et al. (1991) could be due to the vibratory movements of the elastic ear canal walls. This low frequency resonance was so dominating that it could have reduced the possibility of measuring the higher frequency middle ear resonance (RF2) accurately. The present study provided evidence that the magnitudes of the effects as shown by partial eta squared values for RF1 (0.48) and ΔSPL1 (0.31) are higher than for RF2 (0.14) and ΔSPL2 (0.03), respectively (Table 4.3). This indicates the dominant effect of the resonance of the outer ear over the resonance of the middle ear in young infants. In stark contrast to young infants, adults showed a different SFI result pattern which demonstrated the sole dominance of the resonance of the middle ear at about 1000 - 1200 Hz (Wada et al., 1989; Wada et al., 1998).

The present study showed that the mean resonance frequency of the outer ear (RF1) increased from 279 Hz at birth to 545 Hz at 4 months of age. Interestingly, the average mobility of the outer ear (ΔSPL1) decreased from 7.9 dB at birth to 3.7 dB at 4 months (Figure 4.2). As shown in Table 4.2, the resonance of the outer ear (RF1) could be measured in only 73 % of ears (16 out of 22 ears) by 4 months of age and none by 6 months of age. These results illustrate a fast maturation process of the outer ear during the first 6 months of life and RF1 and ΔSPL1data could be used as developmental marker for ear canal development. At birth, the ear canal is relatively flaccid and collapsed (Sprague et al., 1985; Holte et al., 1991; Keefe et al., 1992). As the neonate grows, the anatomy of the outer ear changes with age. In addition to the change in size and mass of the various parts of the outer and middle ear, ossification of the inner two-thirds of the ear canal, change in orientation and fibre structure of the ear drum, and fusion of the tympanic ring are contributing to increased stiffness of the outer ear system.

The SFI findings further suggest that the ossification of the ear canal might be complete by 6 months with negligible ear canal wall movements because by 6 months of age, the resonance of the outer ear disappeared (i.e., RF1 could not be measured). This finding is in agreement with the developmental changes which influence WAI findings as reviewed by Kei et al. (2013). Studies investigating the developmental characteristics of wideband reflectance found that wideband reflectance in the low frequencies (220 - 700 Hz) increased with age during the first 6 months of life due to increasing stiffness of the outer/middle ear system (Sanford & Feeney, 2008; Kei et al., 2013; Aithal et al., 2014; Shahnaz et al., 2014; Hunter et al., 2015).

While the mean resonance frequency of the outer ear (RF1) which has been attributed to the resonance movements of the neonatal ear canal wall (Keefe et al., 1993; Murakoshi et al., 2013) increased with age, no such trend of increasing or decreasing mean resonance of the middle ear (RF2) with age was observed except between 1and 6 months, and 4 and 6 months (Table 4.4). The mean RF2 at 1 month (1373 Hz) and 4 months (1395 Hz) were significantly higher than mean RF2 at 6 months (1174 Hz). This variation could be due to small sample size. In general, the mean middle ear resonance (RF2) for neonates across the first 6 months ranged between 1174 Hz and 1395 Hz, which fell within the previously reported normative range of 830 – 1620 Hz for newborns (Aithal et al., 2014; Aithal et al., 2015) (Table 4.2). It is important to note that the mean resonance frequency for infants from birth (1224 Hz) to 6 months (1174 Hz) in the present study was higher compared to children (1153Hz) and adults (1135 Hz) (Margolis & Goycoolea, 1993; Hunter & Margolis, 1997). However, in the present study, the mean RF2 for the 6-month-old group was low, compared to that for the other age groups. The reasons for the significantly low mean RF2 remain unclear. Perhaps, the variations in RF2 across the age groups may be due to the small sample size of the present study. Similarly, no clear trend of change of the mean ΔSPL2 with age was observed. This supports the previous research findings that the resonance of the middle ear is stable and adult-like at birth (Murakoshi et al., 2013; Aithal et al., 2014). In view of these results, age-specific normative RF2 data would not be necessary. Although RF2 for infants in the present study was stable and higher than adults and children, the range for infants (830 - 1620 Hz) using SFI technique (Aithal et al., 2014; Aithal et al., 2015) overlapped with the range for adults (630 – 1710 Hz) using the SFI technique (Wada et al., 1998) and children (850 – 1525 Hz) using the MFT technique (Hunter & Margolis, 1997).

4.6.1 Limitations

The difficulty of completing the entire battery of tests increased with age. Most of the older infants were tested while they were awake. In order to reduce jaw and suckling movements, infants were not tested when they were crying or restless. Despite all the efforts and time spent on testing older infants, the sample size was still too small for appropriate statistical analysis. While the inclusion of both ears of some infants in the analysis may violate the principle of independence when an ANOVA was applied, the impact of this factor on the outcomes of the study could have minimal effect on the developmental trend of SFI results as most often only one ear from most older infants (> 1 month) was included in the analysis.

The use of a test battery approach in this study necessitated the use of different probes for each test, which disturbed some infants and prolonged the testing time. Further research is needed using an equipment that allows all tests to be completed using a single probe design. Furthermore, the testing time for completing the SFI measures for all ear canal static pressure levels was too long especially when testing older infants who were more wriggly than newborns. To shorten the testing time in future large scale studies, we would suggest to perform SFI testing at ambient pressure and TPP only because the SFI results obtained at other static pressures do not contribute directly to achieve the aim of the present study.

Another potential disadvantage of the SFI technique is the need for repeated sweeps as the pressure is adjusted in steps. SFI testing requires multiple pressurizations, which may not be desirable in the neonate and infant ears, as pressure changes can be a source of discomfort and require maintaining a probe seal for repeated sweeps. This may cause changes in the properties of ear canal and middle ear between pressure sweeps. Since each sweep is essentially an independent measure, the reliability of the relative measures (comparing SPL curves across different sweeps) may be affected if a hermetic seal cannot be maintained, hence introducing artefacts which can affect interpretation of results. However, this can be overcome by measuring the loss of pressure before and after the sweep.

The present study used a cross-sectional study design, which might have restricted, to some extent, the investigation of maturation of the outer and middle ear, especially during the fast developmental period from birth to 4 months. Moreover, 5-month-old infants were not

tested in this study. Hence, it is not clear if 5-month-old infants show SFI findings consistent with the trend of the resonance frequency and mobility observed in the present study. The sample size was small for 2- month and 4- month old infants. Further studies are recommended using a longitudinal study design to track developmental changes of the outer and middle ear using the SFI technique.

4.7 Conclusions

The present study described the developmental characteristics of SFI measures in healthy young infants. There is clear evidence of maturation of the outer ear whereby the resonance frequency increased and mobility decreased with age, indicating increasing stiffness of the outer ear with age consistent with the known anatomical change in ear canal ossification. In contrast, the SFI results did not show significant changes in resonance frequency and mobility of the middle ear, perhaps due to the dominance of the resonance of the outer ear over that of the middle ear. The preliminary normative SFI data developed in this study for the different age groups may potentially be used as a reference for clinical evaluation of the function of the conductive pathway (outer and middle ear) in young infants.

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4.9 References

Aithal S., Aithal V., Kei J. & Driscoll C. 2012. Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia. *J. Am. Acad. Audiol.*, 23, 1-13.

Aithal S., Kei J. & Driscoll C. 2014. Wideband reflectance in young infants (0-6 months): A cross-sectional study. *J. Am. Acad. Audiol.*, 25, 471-478.

Aithal S., Kei J., Driscoll C. & Khan A. 2013. Normative wideband reflectance measures in healthy neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 77, 29-35.

Aithal V., Kei J., Driscoll C., Murakoshi M. & Wada H. 2016. Effects of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns. *Int. J. Pediatr. Otorhinolaryngol.*, 82, 64-72.

Aithal V., Kei J., Driscoll C., Swanston A., Murakoshi M., et al. 2015. Sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 79, 1024-1029.

Aithal V., Kei J., Driscoll C., Swanston A., Roberts K., et al. 2014. Normative sweep frequency impedance measures in healthy neonates. *J. Am. Acad. Audiol.*, 25, 343-354.

Anson B.J. & Donaldson J.A. 1981. *Surgical Anatomy of the Temporal Bone and Ear*. Philadelphia (PA): Saunders.

Baldwin M. 2006. Choice of probe tone and classification of trace patterns in tympanometry undertaken in early infancy. *Int. J. Audiol.*, 45, 417-427.

Calandruccio L., Fitzgerald T.S. & Prieve B.A. 2006. Normative multifreqeuncy tympanometry in infants and toddlers. *J. Am. Acad. Audiol.*, 17, 470-480.

Driscoll C., Kei J. & McPherson B. 2000. Transient evoked otoacoustic emissins in six year old school children: A normative study. *Scand. Audiol.*, 29, 103-110.

Feeney M.P. & Sanford C.A. 2008. Middle-ear measurement in infants and children. *In:* J.R. Madell & C. Flexer (eds.) *Pediatric Audiology: Diagnosis, Technology, and Management*. New York: Thieme, pp. 115-112.

Hamanishi S., Murakoshi M., Steele C., Wada H. & Puria S. 2015. A hybrid ear canal and middle ear model approach for neonates. *Assoc. Res. Otolaryngol*, Abs., PS 145.

Holte L., Cavanaugh R.M. & Margolis R.H. 1990. Ear canal wall mobility and tympanometric shape in young infants. *J. Pediatr.*, 117, 77-80.

Holte L., Margolis R.H. & Cavanaugh R.M. 1991. Developmental changes in multifrequency tympanograms. *Audiology*, 30, 1-24.

Hunter L.L. & Blankenship C.M. 2017. Middle ear measurement. *In:* A. Tharpe, M. & R. Seewald (eds.) *Comprehensive handbook of pediatric audiology*. San Diego: Plural Publishing.

Hunter L.L., Feeney M.P., Miller J.A.L., Jeng P.S. & Bohning S. 2010. Wideband reflectance in newborns: normative regions and relationship to hearing-screening results. *Ear. Hear.*, 31, 599-610.

Hunter L.L., Keefe D.H., Feeney M.P., Fitzpatrick D.F. & Lin L. 2015. Longitudinal development of wideband reflectance tympanometry in normal and at-risk infants. *Hear. Research.*, In press.

Hunter L.L. & Margolis R.H. 1992. Multifrequency tympanometry: current clinical application. *Am. J. Audiol.*, 1, 33-43.

Hunter L.L. & Margolis R.H. 1997. Effects of tympanic membrane abnormalities on auditory function. *J. Am. Acad. Audiol.*, 8, 431 - 446.

Ikui A., Sando I., Haginomori S. & Sudo M. 2000. Postnatal development of the tympanic cavity: A computer-aided reconstruction and measurement study. *Acta. Otolaryngol.*, 120, 375-379.

Keefe D.H. 2008 Wideband measures of middle-ear functioning. *J. Acoust. Soc. Am.*, 13, 5-6.

Keefe D.H., Bulen J.C., Arehart K.H. & Burns E.M. 1993. Ear canal impedance and reflection coefficient in human infants and adults. *J. Acoust. Soc. Am.*, 94, 2617-2638.

Keefe D.H., Folsom R.C., Gorga M.P., Vohr B.R., Bulen J.C., et al. 2000. Identification of neonatal hearing impairment: ear-canal measurements of acoustic admittance and reflectance in neonates. *Ear. Hear.*, 21, 443-461.

Keefe D.H., Hunter L.L., Feeney M.P. & Fitzpatrick D.F. 2015. Procedures for ambient - pressure and tympanometric tests of aural acoustic reflectance and admittance in human infants and adults. *J. Acoust. Soc. Am.*, 138, 3625 - 3653.

Keefe D.H. & Levi E. 1996. Maturation of the middle and external ears: acoustic power-based responses and reflectance tympanometry. *Ear. Hear.*, 17, 361-373.

Keefe D.H., Ling R. & Bulen J.C. 1992. Method to measure acoustic impedance and reflection coefficient. *J. Acoust. Soc. Am.*, 91, 470-485.

Kei J., Allison-Levick J., Dockray J., Harrys R., Kirkegard C., et al. 2003. High-frequency (1000 Hz) tympanometry in normal neonates. *J. Am. Acad. Audiol.*, 14, 20-28.

Kei J., Sanford C.A., Prieve B.A. & Hunter L.L. 2013. Wideband acoustic immittance measures: developmental characteristics (0 to 12 months). *Ear. Hear.*, 34, 17S-26S.

Kei J. & Zhao F. 2012. The challenge of assessing middle ear function in young infants: Summary and future directions. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 163-177.

Kuypers L.C., Decraemer W.C. & Dirckx J.J. 2006. Thickness distribution of fresh and perserved human eardrums measured with confocal microscopy. *Otol. Neurotol.*, 27, 256-264.

Margolis R.H., Bass-Ringdahl S., Hanks W.D., Holte L. & Zapala D.A. 2003. Tympanometry in newborn infants- 1 kHz norms. *J. Am. Acad. Audiol.*, 14, 383-392.

Margolis R.H. & Goycoolea M.V. 1993. Multifrequency tympanometry in normal adults. *Ear. Hear.*, 14, 408-413.

Margolis R.H., Van Camp K.J., Wilson R.H. & Creten W.L. 1985. Multifrequency tympanometry in normal ears. *Audiology*, 24, 44-53.

Mazlan R., Kei J. & Hickson L. 2009. Test-retest reliability of the acoustic stapedial test in healthy neonates. *Ear. Hear.*, 30, 295-301.

Merchant G.R., Horton N.J. & Voss S.E. 2010. Normative reflectance and transmittance measurements on healthy newborn and 1-month-old infnats. *Ear. Hear.*, 31, 1-9.

Meyer S.E., Jardine C.A. & Deverson W. 1997. Developmental changes in tympanometry: A case study. *Br. J. Audiol*, 31, 189-195.

Murakoshi M., Yoshida N., Sugaya M., Ogawa Y., Hamanishi S., et al. 2013. Dynamic characteristics of the middle ear in neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 77, 504-512.

Murakoshi M., Zhao F. & Wada H. 2012. Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 107-129.

Palva T., Northrop C. & Ramsay H. 1999. Spread of amniotic fluid cellular content within the neonate middle ear. *Int. J. Pediatr. Otorhinolaryngol.*, 48, 143-153.

Purdy S.C. & Williams M.J. 2002. High freqeuncy tympanometry: A valid and reliable immittance test protocol for young infnats. *N Z. Audiolog. Soc. Bull*, 10, 9-24.

Qi L., Funnell W.R. & Daniel S.J. 2008. A nonlinear finite-element model of the newborn middle ear. *J. Acoust. Soc. Am.*, 124, 337-347.

Qi L., Liu H., Lutfy J., Funnell W.R. & Daniel S.J. 2006. A nonlinear finite-element model of the newborn ear canal. *J. Acoust. Soc. Am.*, 120, 3789-3798.

Rhodes M.C., Margolis R.H., Hirsch J.E. & Napp A.P. 1999. Hearing screening in the newborn intensive care nursery: comparison of methods. *Otolaryngol. Head. Neck. Surg.*, 120, 799-808.

Roberts D.G., Johnson C.E., Carin S.A., Turczyk V., Karnuta M.A., et al. 1992. Resolution of middle ear effusion in newborns. *Arch. Pediatr. Adolesc. Med.*, 149, 873-877.

Rosowski J.J., Stenfelt S. & Lilly D. 2013. An overview of wideband immittance measurements techniques and terminology: You say absorbance, I say reflectance. *Ear. Hear.*, 34, 9s-16s.

Ruah C.B., Schachern P.A., Zelterman D., Paperella M. & Yoon T. 1991. Age related morphologic changes in the human tympanic membrane. A light and electron microscopic study. *Arch. Otolaryngol. Head. Neck. Surg.*, 117, 627-634.

Sanford C.A. & Feeney M.P. 2008. Effects of maturation on tympanometric wideband acoustic transfer functions in human infants. *J. Acoust. Soc. Am.*, 124, 2106-2122.

Sanford C.A., Keefe D.H., Liu Y.-W., Fitzpatrick D.F., McCreery R.W., et al. 2009. Sound-conduction effects on Distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry. *Ear. Hear.*, 30, 635-652.

Saunders J.C., Kaltenback J.A. & Relkin E.M. 1983. The structural and functional development of the outer and middle ear. *In:* R. Romand & M.R. Romand (eds.) *Development of Auditory and Vestibular systems*. New York: Academic Press, pp. 3-25.

Shahnaz N. 2008. Wideband reflectance in neonatal intensive care units. *J. Am. Acad. Audiol.*, 19, 419-429.

Shahnaz N., Cai A. & Qi L. 2014. Understanding the developmental course of the acoustic properties of the human outer and middle ear over the first 6 months of life by using a longitudinal analysis of power reflectance at ambient pressure. *J. Am. Acad. Audiol.*, 25, 495-511.

Sprague B.H., Wiley T.L. & Goldstein R. 1985. Tymapnometric and acoustic reflex studies in neonates. *J. Speech. Hear. Res.*, 28, 265-272.

Wada H. & Kobayashi T. 1990. Dynamical behavior of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus. *J. Acoust. Soc. Am.*, 87, 237-245.

Wada H., Kobayashi T., Suetake M. & Tachizaki H. 1989. Dynamic behaviour of the middle ear based on sweep frequency tympanometry. *Audiology*., 28, 127-134.

Wada H., Koike T. & Kobayashi T. 1998. Clinical applicability of the sweep frequency measuring appartus for diagnosis of middle ear diseases. *Ear. Hear.*, 19, 240-249.

Wada H., Ohyama K., Kobayashi T., Sunaga N. & Koike T. 1993. Relationship between evoked otoacoustic emissions and middle-ear dynamic characteristics. *Audiology*., 32, 282-292.

Weatherby L.A. & Bennett M.J. 1980. The neonatal acoustic reflex. *Scand. Audiol.*, 9, 103-110.

Werner L.A., Levi E.C. & Keefe D.H. 2010. Ear-canal wideband acoustic transfer functions of adults and two- to nine-month-old infants. *Ear. Hear.*, 31, 587-598.

Zhao F., Wada H., Koike T., Ohyama K., Kawase T., et al. 2003. Transient evoked otoacoustic emissions in patients with middle ear disorders. *Int. J. Audiol.*, 42, 117-131.

Zhao F. & Wang J. 2012. Assessing middle ear function in humans using multifrequency tympanometry: An overview. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 87-106.

Chapter 5: Sweep Frequency Impedance measures in Australian Aboriginal and Caucasian neonates

5.1 Background

Studies based on universal newborn hearing screening program referrals in Australia have shown that prevalence of conductive hearing loss is twice as high in Australian Aboriginal infants (35.19%) compared to Caucasian infants (17.83%) (Aithal et al. 2012). Otitis media has been reported as the main cause of hearing loss (conductive) in this population. Despite high prevalence of otitis media in Australian Aboriginal children, the acoustic-mechanical properties of their outer and middle ear during the neonatal period remain obscured. This study compares the status of the conductive mechanism in 40 ears of 24 Australian Aboriginal neonates with 160 ears of 119 Caucasian neonates using SFI measures. Results comparing SFI data between Australian Aboriginal and Caucasian neonates are presented in Chapter 5 of this thesis.

Chapter 5 of thesis, entitled "Sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates", is based on the manuscript published in the *International Journal of Pediatric Otorhinolaryngology*. This paper is inserted into this thesis with minor modifications. Only formatting of section sub-headings and numbering of tables and figures have been modified from the original publication to match the thesis format. The referencing format of the paper is retained as per the journal format.

Aithal, V., Kei, J., Driscoll, C., Swanston, A., Murakoshi, M., & Wada, H. (2016). Sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates. ***International Journal of Pediatric Otorhinolaryngology,*** **79, 1024-1029. DOI:10.1016/j.ijporl.2015.04.017**

5.2 Abstract

Objective: Despite high prevalence of otitis media in Aboriginal children, the acoustic-mechanical properties of their outer and middle ear during the neonatal period remain obscured. The objective of this study was to compare the acoustic-mechanical properties of

outer and middle ear using Sweep Frequency Impedance (SFI) measures between Australian Aboriginal and Caucasian neonates.

Methods: SFI data from 40 ears of 24 Aboriginal neonates (16 males, 8 females) with mean gestational age of 39.57 wk (SD = 1.25 wk) and 160 ears of 119 Caucasian neonates (57 males, 62 females) with mean gestational age of 39.28 wk (SD = 1.25 wk) serving as controls were analysed. SFI data in terms of resonance frequency (RF) and mobility of the outer and middle ear (ΔSPL) were collected from neonates who passed a test battery that included automated auditory brainstem response, distortion product otoacoustic emissions test and 1000-Hz tympanometry. SFI data were analysed using descriptive statistics and analysis of variance.

Results: There was no significant difference in mean gestational age, age of testing and birth weight between the Aboriginal and Caucasian neonates. The mean resonance frequencies for the outer ear (mean RF1= 264.9 Hz, SD = 58.6 Hz) and middle ear (mean RF2 = 1144 Hz, SD = 228.8 Hz) for Aboriginal neonates were significantly lower than that of Caucasian neonates (mean RF1 = 295.3 Hz, SD = 78.4 Hz and mean RF2 = 1241.8 Hz, SD = 216.6 Hz). However, no significant difference in the mobility of outer ear (ΔSPL1) and middle ear (ΔSPL2) between the two groups was found. Middle ear resonance was absent in 22.5% (9 ears) of Aboriginal ears but present in all Caucasian ears.

Conclusions: This study provided evidence that despite passing the test battery, Aboriginal neonates had significantly lower resonance frequencies of the outer and middle ear than Caucasian neonates. Furthermore, 22.5% of Aboriginal neonates showed no middle ear resonance, indicating the possibility of subtle middle ear issues not detected by the test battery. Reasons for the different acoustic-mechanical properties between the two ethnic groups remain unclear and require further investigation.

Keywords: Neonates, Middle ear, Sweep frequency impedance measure, Acoustic-mechanical properties, Aboriginal, Caucasian

5.3. Introduction

Australian Aboriginal children have a high prevalence of otitis media (OM) compared to Caucasian children [1-6]. OM is reported to start within the first few months of life in Aboriginal infants and children. In a prospective otoscopic study of young infants in 3 Aboriginal communities, Rebetz et al [1] and Douglas and Powers [4] found that, by one year, up to two thirds of infants had at least one perforated ear drum. Peak incidence of ear drum perforation occurred at around 18 weeks and 50 weeks. In another study, Foreman [5] found that of 425 ears examined in Aboriginal infants and young children, only 5 ears (1.2%) were normal and 420 ears (98.8%) had evidence of abnormality.

In a longitudinal study, Boswell and Nienhuys [3] used pneumatic otoscopy, 226 Hz tympanometry and auditory brainstem response audiometry (ABR) to detect OM in 30 Aboriginal infants and 16 Caucasian infants. They reported that 95% of Aboriginal infants compared to only 30% of Caucasian infants showed signs of OM with acute infection by eight weeks after birth. They also reported that once OM started early in life, it became persistent despite treatment in Aboriginal infants.

In another longitudinal study, Lehmann et al [7] monitored middle ear function in 100 Aboriginal and 180 Caucasian infants from birth to two years of age using transient evoked otoacoustic emission (TEOAE) test, 226-Hz tympanometry and otoscopic examinations by an otolaryngologist. They found that TEOAEs were present in 90% (46/51) of Aboriginal and 99% (120/121) of Caucasian neonates aged less than one month. However, the percentage of TEOAEs present dropped to 62% (21/34) for Aboriginal and 93% (108/116) for Caucasian infants aged 1-2 months. These authors also noted that Aboriginal infants who failed TEOAEs at age 1-2 months were 2.6 times more likely to develop OM subsequently than those who passed. However, such prediction was not demonstrated in Caucasian infants with a failed TEOAE outcome at age 1-2 months [7].

In a recent study, Aithal et al [15] studied 211 infants (54 Aboriginal, 157 Caucasian) referred through a newborn hearing screening program in Queensland, Australia. They reported higher prevalence of middle ear pathology in Aboriginal infants (44.4%) compared to Caucasian infants (28.7%). They also reported significantly higher prevalence of conductive hearing loss in Aboriginal infants (37.9%) compared to Caucasian infants (17.8%). Additionally, Aboriginal infants showed poor resolution of conductive hearing loss

over time with 66.7% of Aboriginal infants reviewed showing persistent conductive hearing loss compared to only 17.9% of Caucasian infants.

In summary, the studies on Aboriginal infants have indicated that they are more likely to have OM during the neonatal period and that they are more likely to have recurrent OM later in life compared to their Caucasian peers. The findings of these studies were derived from standard tests which included otoscopy, 226-Hz tympanometry, ABR, 1000-Hz tympanometry (HFT) and TEOAE test. Nevertheless, these tests do not provide detailed information about the acoustic-mechanical properties of outer and middle ear in neonates. Sweep frequency impedance (SFI), an advanced technology, has shown promising results in analysing the acoustic-mechanical behaviour of outer and middle ear in normal neonates [8, 9]. In view of the high prevalence of OM and conductive hearing loss in Aboriginal infants during the first few months of life, it is very important to study the acoustic-mechanical properties of the outer and middle ear system in these neonates.

SFI measures the resonance frequency (RF) and mobility of the outer and middle ear in terms of changes in sound pressure level (ΔSPL) [10-12]. According to Murakoshi et al [9], the resonance that occurs in the low-frequency region (e.g., 250–300 Hz) may be associated with the movement of the elastic external ear canal wall of neonates, while the resonance that occurs in the higher frequency region (e.g., 1100-1300 Hz) may be associated with the movement of the middle ear components. These acoustic-mechanical properties have the potential to detect outer and middle ear dysfunction in neonates.

Nonetheless, to date, there have been no studies that have investigated differences in the acoustic-mechanical properties of the outer and middle ear system between Aboriginal and Caucasian neonates using SFI measures. The research question is: Are there any significant differences in the acoustic-mechanical properties of the outer and middle ear between Aboriginal and Caucasian neonates? The objective of the present study was to compare SFI findings measured at ambient pressure between Australian Aboriginal and Caucasian neonates who passed a test battery containing HFT, distortion product otoacoustic emission (DPOAE), and automated auditory brainstem response audiometry (AABR) screening tests.

5.4 Methods

5.4.1 Participants

The present study included 24 Aboriginal and 119 Caucasian neonates who passed all three tests in a test battery that consisted of AABR, DPOAE and HFT. All neonates had uneventful birth history with no medical complications and risk factors for hearing loss [13]. This study was approved by the Human Research Ethical Committee of Townsville Hospital and Health Service and the University of Queensland Behavioural and Social Sciences Ethical Review Committee (Appendix 1). Parents provided written consent for neonates to be included in the research project (Appendix 2).

Table 5.1 Case details of Aboriginal and Caucasian neonates who passed test battery. Results of t-test showed no significant difference in gestational age, age at time of testing, and birth weight.

	Aboriginal	Caucasian	t	df	P value
Number of neonates	24	119			
Males	16	57			
Females	8	62			
Number of ears	40	160			
Right ear	21	84			
Left ear	19	76			
Gestational Age (weeks)					
Mean	39.57	39.28			
SD	1.25	1.25	0.304	141	NS
90% range	36.4 - 41.3	37 - 41			
Age at time of testing (hours)					
Mean	50.49	45.16			
SD	18.10	19.70	0.222	141	NS
90% range	23.2 - 83.2	19 - 85			
Birth weight (grams)					
Mean	3470.00	3484.90			
SD	414.90	470.00	0.885	141	NS
90% range	2643 - 4230	2730 - 4040			

NS = not significant

Table 5.1 shows the case details of Aboriginal and Caucasian neonates who passed the test battery. Data obtained from 40 ears (21 right and 19 left) of 24 Aboriginal neonates (16 males and 8 females) and 160 ears (84 right and 76 left) of 119 Caucasian neonates (57

males and 62 females) were analysed. The results of independent sample t-test showed no significant differences in gestational age [t (141) = 0.304, p>0.05], age at time of testing [t (141) = 0.222, p>0.05], and birth weight [t (141) = 0.885, p>0.05] between Aboriginal and Caucasian neonates.

5.4.2 Procedure

Otoacoustic emissions (OAEs) and AABR are currently used for hearing screening in neonates. However, successful recording of OAEs and AABR require both healthy inner ear and normal or near normal middle ear function. While passing AABR indicates global normal auditory function, AABR is not sensitive to subtle middle ear and cochlear conditions [14, 15]. Hence a pass in AABR screening may not always assure normal middle ear function. Although OAEs are useful for assessing the function of the conductive pathway, the OAE results may be affected by physiologic and ambient noise [16]. HFT or DPOAE test alone does not appear to be effective in detecting middle ear disorders [17]. While use of a single test alone may not be accurate in detecting middle ear disorders, Aithal et al. [18] advocated the use of a battery of tests which may provide greater assurance of an efficient conductive pathway. In the present study, a test battery consisting of AABR, HFT and DPOAE tests was employed to check for conductive conditions. However, it is acknowledged that it is not an ideal gold standard for detecting conductive disorders.

AABR screening was performed first by clinical nurses of the maternity ward as part of state mandated universal newborn hearing screening (UNHS) program. Following the AABR screen, a clinical audiologist performed HFT, DPOAE and SFI tests, in no particular order. The order of testing was altered depending on the activity state of the neonate and the ease with which a hermetic seal could be obtained for the HFT and SFI tests. All tests were conducted in a quiet room in the maternity ward of the Townsville Hospital in the tropical region of north Queensland. The mean ambient noise level in the testing room was less than 40 dB A. Neonates were usually tested after feeding while in natural sleep or in an awake but quiet condition. Without any preferences, the most accessible ear was tested first. All tests were completed on one ear before the second ear was attempted. The second ear was tested if the neonate was well settled and there was adequate time for testing.

AABR screening was performed using an ALGO3 newborn hearing screener (Natus Medical Inc.). Clicks were presented at 35 dB nHL to both ears simultaneously during

testing. A pass or refer result for each ear was automatically recorded by the equipment. Only the ears that passed the AABR screen were included in this study. While passing AABR indicates global normal auditory function, it is not sensitive to subtle middle ear and cochlear conditions [14, 15]. Additional tests are needed to evaluate the function of the periphery auditory system.

HFT was performed using a Madsen Otoflex 100 acoustic immittance device (GN Otometrics) with a 1000 Hz probe tone. Admittance (Ya) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec. Pass criteria were a single positively peaked tympanogram with the middle ear pressure between 50 and -150 daPa and peak compensated static admittance (+200 daPa tail to peak) of at least 0.2 mmho [19, 20].

DPOAE screen was performed using a Biologic Navigator Plus device. DPOAEs were obtained in response to stimulation by pairs of primary tones. The f2/f1 frequency ratio was 1.2 for each primary pair. The level of f1 was 65 dB SPL and f2 was 55 dB SPL. The 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 [17, 21] and (ii) DPOAE amplitude of at least -6 dB at 2, 3, 4 and 6 kHz [17, 22].

SFI test was performed using a new SFI unit developed for testing neonates [9]. The SFI unit and its calibration have been described in detail by Murakoshi et al [9]. Figure 5.1 shows a block diagram of this SFI unit. It consisted of a personal computer, an AD/DA converter, a probe system, a stepping motor, an air pump, a pressure sensor, and a pressure relief valve. The probe consisted of 3 tubes: the first tube to apply static pressure (Ps) to the ear canal, the second tube to deliver sound to the external ear canal via an earphone, and the third tube to measure sound pressure in the external ear canal using a microphone. A specially designed cuff suitable for testing neonates was attached to the tip of the probe to obtain a hermetic seal during testing. This unit was controlled using LabView under MS WINDOWS.

During the SFI test, the sound pressure level in the ear canal was measured as the frequency of the pure tone stimulus was swept from 100 to 2200 Hz while the external auditory canal static pressure (Ps) was held constant at +200 daPa. This measurement was repeated with Ps reduced in 50 daPa steps down to -200 daPa. The entire SFI procedure was

automated and it took less than two minutes to complete the test in each ear. The sweeping probe tone level was kept below 75 dB SPL to reduce the risk of eliciting the acoustic stapedial reflex. While the SFI results measured at multiple static pressures provide a comprehensive view of the acoustic-mechanical properties of the outer and middle ear, Murakoshi et al [9] and Aithal et al [8] found that measurements made at ambient pressure (0 daPa) can provide adequate clinical information about the status of the outer and middle ear. For the purpose of the present study, only measurements performed at ambient pressure were included in the analyses.

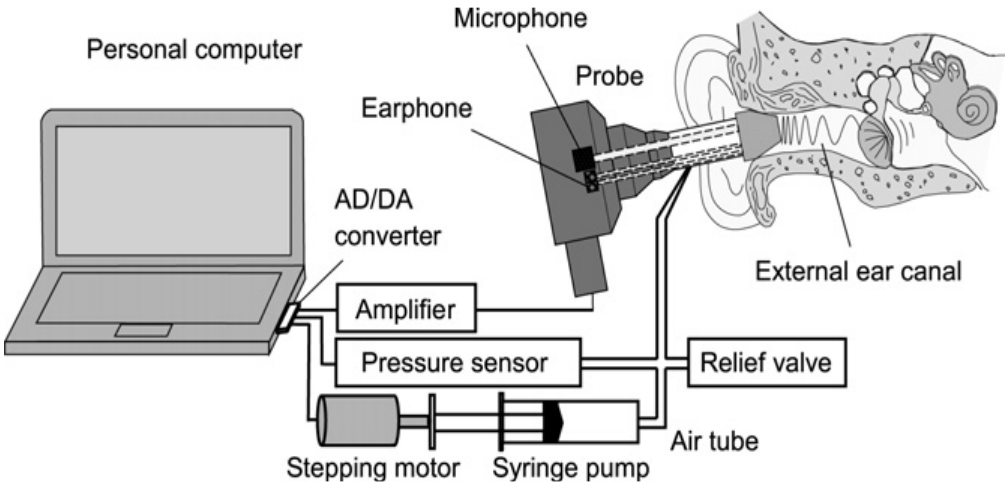

Figure 5.1. Block diagram of SFI metre used to test neonates in this study. The SFI meter consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, a syringe pump, a pressure sensor and a relief valve. This new unit is controlled using LabView under WINDOWS. [From Murakoshi et al (2013). Int J Pediatr Otorhinolaryngol. Copyright © 2012 by Elsevier Ireland Ltd. Reprinted with permission of Elsevier Ireland Ltd.]

5.4.3 Analysis of data

Descriptive statistics are provided for all SFI measures (RF1, RF2, ΔSPL1 and ΔSPL2) for both Aboriginal and Caucasian groups. An analysis of variance (ANOVA) was used to compare SFI measures between the Aboriginal and the Caucasian neonates. The effect of ear (right versus left) and gender (male versus female) on SFI results was evaluated using the ANOVA. An alpha level of 0.05 was used for all statistical procedures.

5.5 Results

Figure 5.2 shows typical SFI results obtained from the left ear of a healthy two-day-old Aboriginal neonate who passed the test battery of AABR, HFT and DPOAE tests. The SPL curve (bold curve) obtained at ambient pressure (0 daPa) shows an increase in SPL at two frequency regions, F_b1 to F_a1 and F_b2 to F_a2 with corresponding SPL at P_b1, P_a1, P_b2 and P_a2, respectively. Previous studies have shown that the greatest variation (displacement) of SPL (ΔSPL) occurs at median frequencies RF1 and RF2, which are halfway between the frequencies F_a1 and F_b1, and between F_a2 and F_b2, respectively [9, 11]. Murakoshi et al [9] identified the first resonance at RF1 which is the frequency at which the SPL varies considerably between F_b1 to F_a1. Hence, RF1 and the corresponding variation in SPL (Δ SPL1) are defined as shown in Equations (5.1) and (5.2) [9, 12].

First Resonance Frequency, $RF1 = (F_a1+F_b1) / 2$ Equation (5.1)

SPL change at RF1, $\Delta SPL1 = P_a1 - P_b1$ Equation (5.2)

Similarly, the second resonance frequency (RF2) and the corresponding variation in SPL (Δ SPL2) are defined as shown in Equations (5.3) and (5.4) [9].

Second Resonance Frequency, $RF2 = (F_a2+F_b2) / 2$ Equation (5.3)

SPL change at RF2, $\Delta SPL2 = P_a2 - P_b2$ Equation (5.4)

As illustrated in the Figure 5.2, when the static pressure (Ps) = 0 daPa (ambient pressure), RF1 = 260 Hz, Δ SPL1 = 12 dB, RF2 = 1200 Hz and Δ SPL2 = 8 dB. These results indicate that there are two distinct resonance frequencies at which the SPL varies considerably. The Δ SPL variation reflects the mobility of the outer and middle ear system at these frequencies [8, 9]. According to Murakoshi et al (2013) and Aithal et al (2014), RF1 and ΔSPL1 are associated with resonance in the outer ear, while RF2 and ΔSPL2 are associated with resonance in the middle ear.

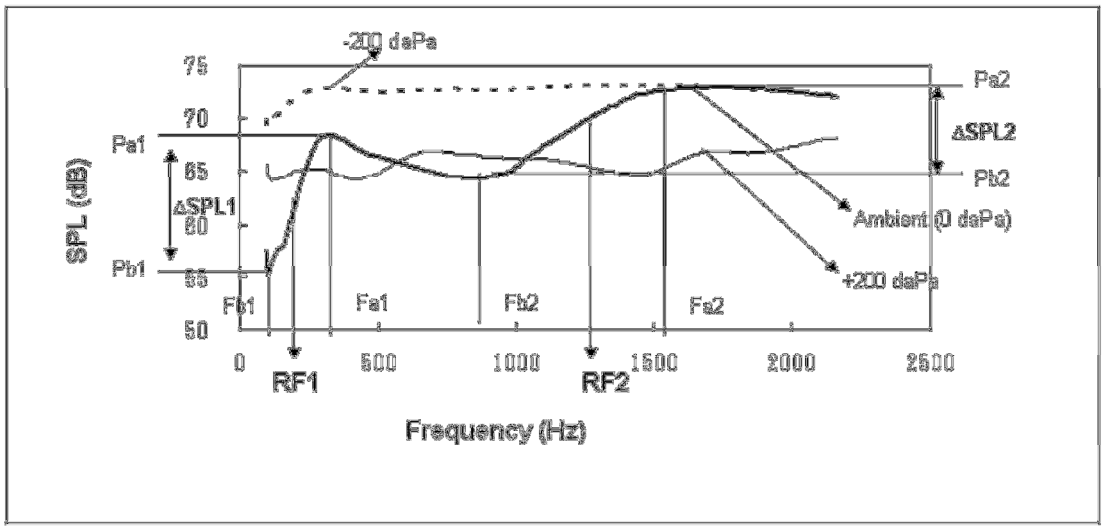

Figure 5.2. A typical SFI results obtained from left ear of a healthy two day-old neonate that passed the test battery. The static ear canal pressure (daPa) applied were +200, 0 (ambient), and -200 daPa. P_a1 and P_b1 are the maximum and minimum sound pressures, and F_a1 and F_b1 are the frequencies corresponding to these sound pressures (first variation). P_a2 and P_b2 are the maximum and minimum sound pressures, and F_a2 and F_b2 are the frequencies corresponding to these sound pressures (second variation). RF1 and RF2 are defined by $(F_a1+F_b1)/2$, and $(F_a2+F_b2)/2$, respectively. ΔSPL1 and ΔSPL2 are defined by $(P_a1- P_b1)$ and $(P_a2 - P_b2)$, respectively.

As the static air pressure (Ps) in the ear canal was increased to +200 daPa, both RF1 and RF2 increased, while both Δ SPL1 and Δ SPL2 decreased indicating an increase in stiffness of outer and middle ear system. This acoustic-mechanical behaviour of the outer and middle ear is typical of the healthy neonate ear. When a negative static pressure of - 200 daPa was applied to the ear canal the SPL curve did not show much variation of SPL with frequency. Instead, a relatively flat response to frequency with an overall sound pressure of 73 dB SPL was observed, suggesting that the ear canal had collapsed [8, 9], showing flat responses, similar to the response observed in a calibration cavity [9].

The SFI result pattern for all 160 Caucasian and only 31 Aboriginal neonate ears in the present study showed normal SFI pattern as shown in Figure 2, consistent with that of other studies [8, 9]. However, the second resonance peak (RF2) was not recorded in 9 Aboriginal neonate ears, despite these neonates passing the test battery. This absence of

second resonance peak (RF2) in Aboriginal ears is consistent with middle ear dysfunction previously reported by Murakoshi et al [12].

Table 5.2 shows a summary of descriptive statistics showing the mean, SD, and 90% range for RF1, RF2, ΔSPL1, and ΔSPL2 for Aboriginal and Caucasian neonates. RF2 and ΔSPL2 could not be identified in 9 ears of Aboriginal neonates. Mean RF1 was 264.9 Hz with a SD of 58.6 Hz and mean RF2 was 1144 Hz with SD of 228.8 Hz for Aboriginal neonates. Mean RF1 was 295.3 Hz with SD of 78.4 Hz and mean RF2 was 1241.8 Hz with SD of 216.6 Hz for Caucasian neonates. The 90% normal range for RF1 was from 180 to 377.5 Hz and RF2 was from 715.5 to 1449.2 Hz for Aboriginal neonates. The normal range for RF1 was from 209 to 460 Hz and RF2 was from 870 to 1619.5 Hz for Caucasian neonates. Mean SPL1 was 7.26 dB with SD of 2.8 dB and SPL2 was 4.4 dB with SD of 2.2 dB for Aboriginal neonates whereas mean SPL1 was 8.14 dB with SD of 3.1 dB and mean SPL2 was 4.9 dB with SD of 2.2 dB for Caucasian neonates.

5.5.1 Within-group analysis

An analysis of variance (ANOVA) was applied separately to RF1, RF2, ΔSPL1, and ΔSPL2 with gender (male versus female) and ear (right versus left) as independent variables for Aboriginal neonates. The results showed no significant gender or ear effects for RF1 [gender: $F(1,36) = 2.25$, $p>0.05$; ear: $F(1,36) = 0.03$, $p>0.05$], RF2 [gender: $F(1,27) = 0.64$, $p>0.05$; ear: $F(1,27) = 1.49$, $p>0.05$], ΔSPL1 [gender: $F(1,36) = 0.03$, $p>0.05$; ear: $F(1,36) = 0.38$, $p>0.05$] and ΔSPL2 [gender: $F(1,27) = 3.27$, $p>0.05$; ear: $F(1,27) = 0.00$, $p>0.05$]. There was no significant Ear × Gender interaction.

The above ANOVA analysis was also applied to the data obtained from Caucasian neonates. The results showed no significant gender or ear effects for RF1 [gender: $F(1,156) = 0.13$, $p>0.05$; ear: $F(1,156) = 0.94$, $p>0.05$], RF2 [gender: $F(1,156) = 0.53$, $p>0.05$; ear: $F(1,156) = 0.77$, $p>0.05$], ΔSPL1 [gender: $F(1,156) = 0.63$, $p>0.05$; ear: $F(1,156) = 0.03$, $p>0.05$] and ΔSPL2 [gender: $F(1,156) = 0.01$, $p>0.05$; ear: $F(1,156) = 3.76$, $p>0.05$]. There was no significant interaction between ear and gender.

Table 5.2 Mean, SD, 90% range for different SFI measures (RF1, RF2, ΔSPL1 and ΔSPL2) for Aboriginal and Caucasian neonates. RF2 and ΔSPL2 could not be identified for 9 ears of Aboriginal neonates.

	Aboriginal Neonates	Caucasian Neonates
RF1		
n (ears)	40	160
Mean (Hz)	264.9	295.3
SD (Hz)	58.6	78.4
90% range (Hz)	180 - 377.5	209 - 460
RF2		
n (ears)	31	160
Mean (Hz)	1144	1241.8
SD (Hz)	228.8	216.6
90% range (Hz)	715.5 - 1449.2	870 - 1619.5
ΔSPL1		
n (ears)	40	160
Mean (dB)	7.26	8.14
SD (dB)	2.8	3.1
90% range (dB)	2.3 - 12	2.8 - 13.5
ΔSPL2		
n (ears)	31	160
Mean (dB)	4.4	4.9
SD (dB)	2.2	2.2
90% range (dB)	1.4 - 8.9	1.2 - 8.4

5.5.2 Between-group analysis

To investigate the possibility of an ethnic effect on the SFI measures, an ANOVA was applied separately to RF1, RF2, ΔSPL1 and ΔSPL2 with ethnicity (Aboriginal versus Caucasian) as an independent variable. The effect of ethnicity was significant for both RF1 [$F(1,198) = 5.28$, $p = 0.02$] and RF2 [$F(1,189) = 5.19$, $p = 0.02$] with the Aboriginal neonates having significantly lower RF1 and RF2 values than those of the Caucasian neonates (Table 5.2). Power analysis (partial eta squared) showed 0.03 magnitude of effect for RF1 and RF2

(Table 5.3) which fell between small (0.01) and medium (0.09) effect. Table 5.3 shows the between-group ANOVA results of SFI measures obtained from Aboriginal and Caucasian neonates.

Table 5.3 Between-group (Aboriginal versus Caucasian) ANOVA results of SFI measures obtained from Aboriginal and Caucasian neonates

	F Value	df	P value	Observed Power	Partial Eta Squared
RF1	5.28	1	0.02*	0.63	0.03
Error		198			
RF2	5.19	1	0.02*	0.62	0.03
Error		189			
Δ SPL1	2.64	1	0.11	0.37	0.01
Error		198			
Δ SPL2	1.62	1	0.20	0.25	0.01
Error		189			

* = significant with $p < 0.05$

To understand the application of group-specific norm on overall test performance, further analysis was performed using false alarm (FA) rate. A false alarm rate was calculated in the Aboriginal group based on the normative 90% range obtained from the Caucasian neonates. As we do not have large number of confirmed disorders of outer and middle ear condition in Aboriginal group, it was not possible to determine the sensitivity index. Nevertheless, it was possible to determine specificity index using present data. Establishing specificity index is important as specific test rarely results in false alarms. A positive result on a specific test is a good indicator for a disease condition.

The 5th percentile of RF1 and RF2 for Caucasian group (Table 5.2) was used as a cut-off to determine the FA rate for the Aboriginal group. The 95th percentile of RF1 and RF2 for Caucasian group will not alter any FA rate because this value is much higher than it is in the Aboriginal group. If the two ethnic groups have identical acoustic-mechanical properties, the

5th percentile in the Caucasian group should result in a 5% FA rate in the Aboriginal group. However, application of the 5th percentile Caucasian cut-off resulted in 12.5% FA rate for RF1 and 15% FA rate for RF2 in the Aboriginal neonates.

5.6. Discussion

The present study compared the acoustic-mechanical properties of the outer and middle ear in Australian Aboriginal and Caucasian neonates who passed AABR, HFT and DPOAE using SFI measures. Aboriginal neonates had significantly lower mean RF1 compared to Caucasian neonates group (264.9 Hz versus 295.3 Hz, see Table 5.2). The Aboriginal group also had smaller 90% range than Caucasian group (180 to 377.5 Hz versus 209 to 460 Hz). Furthermore, both the 5th and 95th percentiles of Aboriginal neonates were lower than those of the Caucasian neonates. These results showed that Aboriginal neonates who passed the test battery had significantly different acoustic-mechanical properties than their Caucasian counterparts (Tables 5.2 and 5.3).

The reasons for the difference in RF1 between Aboriginal and Caucasian neonates are not clear. Perhaps, there are possible dissimilarities in anatomical structure and physiological function of the outer and middle ear system between the two groups. At birth, the neonate ear canal is relatively flaccid and prolapsed [23-25]. It is possible that Aboriginal neonates may have more flaccid and prolapsed ear canal than Caucasian neonates, resulting in reduced resonance frequency of the outer ear (RF1) compared to that of the Caucasian neonates. Another reason for reduced RF1 in Aboriginal neonates could be due to the increased mass of their outer ear due to the presence of vernix, a waxy substance that covers the skin of the newborn ear canal [26-28]. However, no studies have been performed to support the proposition that the outer ear of Aboriginal neonates has more vernix than that of Caucasian neonates.

The contributing factors to the decreased middle ear resonance frequency (RF2) in Aboriginal neonates are not confirmed. It could be a direct result of the increased mass load and or decreased stiffness on the middle ear system due to middle ear effusion (MEE) [21, 29, 30], presence of materials other than air in the middle ear cavity such as amniotic fluid or mucoid effusions [31], mesenchymal tissue in the middle ear space [32], or loss of stiffness due to decreased air in the middle ear.

There is further evidence to support the above proposition. The results of the present study showed that although all ears passed the battery of tests (AABR, HFT and DPOAE), nine Aboriginal ears did not show the second resonance (RF2) whereas all Caucasian ears demonstrated the presence of RF2 (Table 5.2). As suggested by Murakoshi et al [9], the second resonance relates to the acoustic-mechanical properties of the middle ear. The absence of a second resonance in SFI findings may indicate middle ear dysfunction [12]. It is possible that SFI demonstrated compromised acoustic-mechanical properties of the middle ear of Aboriginal neonates which could not be identified by the battery of tests [33]. Furthermore, the fluid and other materials affecting the middle ear could have altered the acoustic-mechanical properties of the middle ear (i.e., RF2 decreased or absent) of Aboriginal neonates more than Caucasian neonates.

The present study found no significant difference in both ΔSPL1 and ΔSPL2 between Aboriginal and Caucasian neonates (Table 5.2). Nevertheless, there was a trend for Aboriginal neonates to have lower ΔSPL1 and ΔSPL2 values than their Caucasian counterparts. As ΔSPL1 and ΔSPL2 relate to the mobility of the outer ear and the middle ear, respectively [9, 12], the reduced ΔSPL1 and ΔSPL2 values of the Aboriginal neonates may indicate less mobility of the outer and middle ear system in Aboriginal neonates than in Caucasian neonates.

In view of above findings, follow up testing of Aboriginal neonates should be arranged to monitor their outer and middle ear function during the infancy period (1 month – 3 years). More research is needed to track the acoustic-mechanical properties of the Aboriginal neonates' peripheral auditory system to determine whether they develop chronic OM later in life. The reduced RF1 and RF2 values of the Aboriginal neonates may indicate possible altered acoustic-mechanical properties of outer and middle ear which may help to predict the development of chronic OM as the neonates grow. This would require a large-scale longitudinal study to test this hypothesis.

As Aboriginal neonates had significantly lower RF1 and RF2, further analysis was performed using false alarm (FA) rate to understand the application of group-specific norm on overall test performance. Application of the 5^{th} percentile Caucasian cut-off point resulted in higher FA rate for both RF1 and RF2 in the Aboriginal neonates. The application of

Caucasian norms to the Aboriginal group almost doubled FA rate for RF1 and tripled FA rate for RF2. This suggests that if the normative data for Caucasian neonates were applied to the Aboriginal neonates, the FA would be increased unnecessarily. Perhaps, a separate set of normative SFI data for the Aboriginal should be used to reduce the FA rate.

5.6.1 Limitations

Although testing was performed when neonates were well settled, the use of multiple probe tips for conducting the HFT, DPOAE and SFI tests disturbed some neonates. Testing had to be discontinued for some neonates who became unsettled due to multiple probe insertion. Improvement in instrumentation to include a single probe assembly to perform multiple tests is therefore recommended. This improvement will also reduce overall testing time and increased the completion rate in testing neonates.

Unlike the HFT, SFI test required multiple pressurizations when a sweeping frequency tone was delivered to the ear. As the SFI test required a tight probe seal for repeated sweeps, pressurization might be a source of discomfort for few neonates. This difficulty was partially overcome by testing neonates when they were asleep.

Another limitation of the present study is related to the obscure clinical relationship between compromised acoustic-mechanical properties and function of the conductive pathway (outer and middle ear) in Aboriginal neonatal ears. The causative factors which may account for the difference in acoustic-mechanical properties of the conductive pathway between Caucasian and Aboriginal neonates remain unclear at this stage. Nevertheless, the results of the present study indicate that the SFI test may be more sensitive to subtle conductive conditions than the test battery (AABR, DPOAE and 1000 Hz tympanometry) in the Aboriginal neonates.

5. 7 Conclusions

The present study demonstrated that Aboriginal neonates who passed the AABR, HFT and DPOAE test battery had significantly different acoustic-mechanical properties of outer and middle ear as evidenced by lower outer and middle ear resonance (RF1 and RF2, respectively) than their Caucasian counterparts. These decreased resonance frequencies of the

outer and middle ear suggest that Aboriginal neonates are more likely to have inferior acoustic-mechanical properties of outer and middle ear at birth than Caucasian neonates.

While it is possible that anatomical and physiological characteristics of the outer and middle ear, between two ethnic groups could have resulted in differences in SFI findings, the contributing factors cannot be confirmed using the present experimental design. Further studies which provide anatomical and physiological data are warranted. It would be useful to conduct longitudinal studies using SFI measures to determine whether Aboriginal neonates with reduced outer and middle ear dynamic function are prone to OM later in life.

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5.9 References

[1] P.T.E. Rebgetz , P.Trennery , J. Powers , J. Mathews , Incidence of otitis media in the first year of life. Menzies School of Health Research Annual Report (1988-89) p.67.

[2] J. Boswell, A Prospective Audiological Study of otitis Media in Aboriginal and Non-Aboriginal Australian Infants, PhD Thesis, University of Sydney, Sydney, 1994.

[3] J. Boswell, T. Nienhuys, Onset of otitis media in the first eight weeks of life in Aboriginal and non-aboriginal Australian infants, Ann. Otol. Rhinol. Laryngol. 104 (1995) 542-549.

[4] F. Douglas, J. Powers, Ear disease in infancy in three rural Aboriginal communities. Menzies School of Health Research Annual Report (1988-89) 68-69.

[5] A.E.W. Foreman, The Aetiology and Prevention of Otitis Media in Aboriginal Children in the Northern Territory, Australia, Thesis submitted for MPH, University of Sydney, Sydney 1987.

[6] Australian Bureau of Statistics (ABS), 4704.0 The Health and Welfare of Australia's Aboriginal and Torres Strait Islander Peoples. In: ABO Statistics (ed), Canberra, 2010.

[7] D. Lehmann, S. Weeks, P. Jacoby, D.Elsbury, J. Finucane, A. Stokes, R.Monck, H. Coates, Absent otoacoustic emissions predict otitis media in young Aboriginal children: a birth cohort study in Aboriginal and non-Aboriginal in an arid zone of Western Australia, BMC Pediatr 8 (2008) 32- 43.

[8] V. Aithal, J. Kei, C. Driscoll, A. Swanston, K. Roberts, M. Murakoshi, et al. Normative sweep frequency impedance measures in healthy neonates, J. Am. Acad. Audiol. 25 (2014) 343-354.

[9] M. Murakoshi, N. Yoshida, M. Sugaya, Y. Ogawa, S. Hamanishi, H. Kiyokawa et al, Dynamic characteristics of the middle ear in neonates, Int. J. Pediatr. Otorhinolaryngol. 77 (2013) 504-512.

[10] H. Wada, T. Kobayashi, Dynamical behaviour of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus, J. Acoust. Soc. Am. 87 (1990) 237-245.

[11] H. Wada, T. Kobayashi, M. Suetake, H. Tachizaki, Dynamic behaviour of the middle ear based on sweep frequency tympanometry, Audiology 28 (1989) 127-134.

[12] M. Murakoshi, F. Zhao, H. Wada, Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. In: J. Kei., F. Zhao (Eds.), Assessing Middle Ear Function in Infants, Plural, San Diego, 2012, pp.107-129.

[13] Joint Committee on Infant Hearing (JCIH), Year 2007 position statement: Principles and guidelines for early hearing detection and intervention programs,. Pediatrics 120 (2007) 898-921.

[14] J. Kei, R. Mazlan, High-frequency tympanometry: Clinical applications. In: J. Kei, F. Zho (Eds.), Assessing Middle Ear Function in Infants, Plural, San Diego, 2012, pp.39-67.

[15] S. Aithal, V. Aithal, J. Kei, C. Driscoll, Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia, J. Am. Acad. Audiol. 23 (2012) 1-13.

[16] C. Driscoll, J. Kei, B. McPherson, Transient evoked otoacoustic emissions in six-year-old school children: a normative study , Scand. Audiol. 29 (2000) 103-110.

[17] C. A. Sanford, D.H. Keefe, Y-W. Liu, D.F. Fitzpatrick, R.W. McCreery, D.E. Lewis, et al., Sound-conduction effects on distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry, Ear Hear. 30 (2009) 635-652.

[18] S. Aithal, J. Kei, C. Driscoll, A. Khan, Normative wideband reflectance measures in healthy neonates, Int. J. Pediatr. Otorhinolaryngol. 77 (2013) 29-35.

[19] R. Mazlan, High frequency (1000 Hz) tympanometry and acoustic reflex findings in newborn and 6-weeks-old infants. PhD Thesis, University of Queensland, Brisbane, 2009.

[20] R. Mazlan, J. Kei, L. Hickson, Test-retest reliability of the acoustic stapedial test in healthy neonates, Ear Hear. 30 (2009) 295-301.

[21] L. L. Hunter, M.P. Feeney, J.A.L. Miller, P.S. Jeng, S. Bohning, Wideband reflectance in newborns: normative regions and relationship to hearing-screening results, Ear Hear. 31 (2010) 599-610.

[22] G. R .Merchant, N. J. Horton, S. E. Voss, Normative reflectance and transmittance measurements on healthy newborn and 1-month-old infants, Ear Hear. 31 (2010) 1-9.

[23] L. Holte, R. H. Margolis, R. M. Cavanaugh, Developmental changes in multifrequency tympanograms, Audiology. 30 (1991) 1-24.

[24] D. H. Keefe, Method to measure acoustic impedance and reflection coefficient, J. Acoust. Soc. Am. 91 (1993) 470-485.

[25] B. H. Sprague, T. L. Wiley, R. Goldstein, Tympanometric and acoustic reflex studies in neonates, J.Speech Lang.Hear.Res. 28 (1985) 265-272.

[26] K. J. Doyle, B. Burggraaff, S. Fujikawa, J. Kim, C. J. Macarthur, Neonatal hearing screening with otoscopy, auditory brainstem response and otoacoustic emissions, Otolaryng. Head Neck. Surg. 116 (1997) 597-603.

[27] T. J Balkany, S. A. Berman, M. A. Simmons, B. W. Jafek, Middle ear effusions in neonates, Laryngoscope 88 (1978) 398-405.

[28] R. M. Cavanaugh, Pneumatic otoscopy in healthy full-term infants, Pediatrics 79 (1987) 520-523.

[29] N. Shahnaz, Clinical application of wideband reflectance (WBR) in infants, children and adults, in: Canadian Hearing Research Report 5 (2010) 23-29.

[30] A. N. Beers, N. Shahnaz, B. D. Westerberg, F. K. Kozak, Wideband reflectance in normal Caucasian and Chinese school-age children and in children with otitis media with effusion, Ear Hear 31 (2010) 221-223.

[31] D. J. deSa, Infection and amniotic aspiration of middle ear in stillbirths and neonatal deaths, Arch. Dis. Child. 48 (1973) 872-880.
[32] M. M. Paparella, D. Shea, W. L. Meyerhoff, M. V. Goycoolea, Silent otitis media, Laryngoscope 90 (1980) 1089-1098.
[33] S. Aithal, J. Kei, C. Driscoll, Wideband Absorbance in Australian Aboriginal and Caucasian Neonates, J. Am. Acad.Audiol. 25 (2014) 482-494.

Chapter 6: Predictive accuracy of Sweep Frequency Impedance technology in identifying conductive conditions in newborns

6.1 Background

Predicting the true status of the outer and middle ear in newborns and young infants is very difficult and challenging. From an audiological perspective, there is no "gold standard" for assessing the function of the conductive pathway in this population. Although the use of air and bone conduction (AC and BC) auditory brainstem response (ABR) threshold assessment using frequency specific tone bursts as stimuli may be used a surrogate gold standard, the ABR test is time consuming and not routinely used in newborn hearing screening programs. Previous studies have used otoacoustic emissions (OAEs) and high frequency tympanometry (HFT) as reference standards for determining the status of the outer and middle ear. However, these single test standards are not sensitive to subtle conductive conditions. The present study is the first attempt to evaluate the predictive accuracy of SFI which is an emerging technology to identify conductive conditions in newborns and young infants.

This study compared the test performance of SFI against 4 commonly used single tests (AABR, HFT, DPOAE and TEOAE) and 5 test batteries (HFT+DPOAE, HFT+TEOAE, DPOAE+TEOAE, DPOAE+AABR and TEOAE+AABR) as reference standards. The purpose of this study was to determine whether the SFI test can provide a more effective alternate reference standard to either individual tests or a combination of tests for determining the status of the outer and middle ear. The results of the study are presented in Chapter 6 of this thesis.

Chapter 6 of this thesis, entitled "Predictive accuracy of Sweep Frequency Impedance technology in identifying conductive conditions in newborns" is based on a manuscript accepted for publication in *Journal of the American Academy of Audiology*. This paper is inserted into this thesis with minor modifications. In particular, only the formatting of section sub-headings and numbering of tables and figures have been modified from the original manuscript to match the thesis format. The referencing format of the paper is retained as per the journal format.

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6.2 Abstract

Background: Diagnosing conductive conditions in newborns is challenging for both audiologists and otolaryngologists. Although high frequency tympanometry (HFT), acoustic stapedial reflex tests, and wideband absorbance (WBA) measures are useful diagnostic tools, there is performance measure variability in their detection of middle ear conditions. Additional diagnostic sensitivity and specificity measures gained through new technology such as sweep frequency impedance (SFI) measures may assist in the diagnosis of middle ear dysfunction in newborns.

Purpose: The purpose of this study was to determine the test performance of SFI to predict the status of the outer and middle ear in newborns against commonly used reference standards.

Research Design: Automated auditory brainstem response (AABR), HFT (1000 Hz), transient evoked otoacoustic emission (TEOAE) and distortion product otoacoustic emission (DPOAE), and SFI tests were administered to the study sample.

Study Sample: A total of 188 neonates (98 males and 90 females) with a mean gestational age of 39.4 weeks. Mean age at the time of testing was 44.4 hours.

Data Collection and Analysis: Diagnostic accuracy of SFI was assessed in terms of its ability to identify conductive conditions in neonates when compared with 9 different reference standards [including 4 single tests (AABR, HFT, TEOAE and DPOAE) and 5 test batteries (HFT+DPOAE, HFT+TEOAE, DPOAE+TEOAE, DPOAE+AABR and TEOAE+AABR)], using receiver operating characteristic (ROC) analysis and traditional test performance measures such as sensitivity and specificity.

Results: The test performance of SFI against the test battery reference standard of HFT+DPOAE and single reference standard of HFT were high with an area under the ROC curve (AROC) of 0.87 and 0.82, respectively. Although the HFT+DPOAE test battery reference standard performed better than the HFT reference standard in predicting middle ear conductive conditions in neonates, the difference in AROC was not significant. Further analysis revealed that the highest sensitivity and specificity for SFI (86% and 88%, respectively) was obtained when compared with the reference standard of HFT+DPOAE. Among the 4 single reference standards, SFI had the highest sensitivity and specificity (76% and 88%, respectively), when compared against the HFT reference standard.

Conclusions: The high test performance of SFI against the HFT and HFT+DPOAE reference standards indicates that the SFI measure has appropriate diagnostic accuracy in detection of conductive conditions in newborns. Hence, the SFI test could be used as adjunct tool to identify conductive conditions in universal newborn hearing screening (UNHS) programs, and can also be used in diagnostic follow up assessments.

Key words: Sweep frequency impedance, resonance frequency, high frequency tympanometry, multifrequency tympanometry, middle ear, newborn, test performance, dynamic behaviour, reference standards, receiver operating characteristics.

Abbreviations:

AABR = automated auditory brainstem response;
AROC = area under receiver operating curve;
daPa = deca Pascal;
dB = decibel;
DPOAE = distortion product otoacoustic emissions;
Δ = delta;
Hz = hertz;
HFT = high frequency tympanometry;
JCIH = Joint committee on infant hearing;
MFT = multifrequency tympanometry;
OAE = otoacoustic emissions;
OME = otitis media with effusion;
pkSPL = peak sound pressure level;

Ps = static pressure;

RF = resonance frequency;

ROC = receiver operating characteristics;

SFI = sweep frequency impedance;

SN = sensorineural;

SPL = sound pressure level;

TEOAE = transient evoked otoacoustic emissions;

TPP = tympanometric peak pressure;

UNHS = universal newborn hearing screening;

WBA = wideband absorbance;

Ya = static admittance;

Ypc = peak compensated static admittance;

6.3 Introduction

Universal newborn hearing screening (UNHS) to detect permanent hearing loss using otoacoustic emissions (OAE) or automated auditory brainstem response (AABR) has become standard practice across many countries. Despite improvements in the screening technology, high rates of referrals due to transient conductive conditions continue to be an issue with UNHS programs. A child who failed the screening test but later identified to have normal hearing is regarded as having a transient conductive hearing loss (Clemens et al., 2000; Clemens & Davis, 2001; Mehl & Thompson, 2002; Keefe & Feeney, 2009). However, some children may be found to have a congenital or long standing conductive condition which requires medical intervention (Boudewyns et al., 2011). Several studies have reported high rates of referrals of 3 to 8% in newborns (Mason & Herrman, 1998; Mehl & Thomson, 1998; Vohr et al., 1998; Clemens et al., 2000; Clemens & Davis, 2001) that is attributed to transient conductive loss due to outer and middle ear dysfunction. As expected, middle ear dysfunction or cochlear hearing loss obliterates OAE and AABR responses, resulting in a "refer" outcome. It is, therefore, not possible to distinguish between a conductive or cochlear hearing loss when a refer outcome occurs (Allen et al., 2005).

In newborns, a transient conductive condition may be due to vernix occluding the ear canal, residual amniotic fluid or mesenchyme in the middle ear space of well neonates (Buch & Jorgensen, 1964; Kok et al., 1992; Keefe et al., 2000; Rosenfeld et al., 2004) and neonates

cared for in the neonatal intensive care unit (NICU) (Balkany et al., 1978; Paradise, 1981; Derkay et al., 1988). Transient outer and middle ear dysfunction is common in neonates tested within 48 hours of birth and infants in NICU for extended periods of time (Keefe & Feeney, 2009). As most newborns are screened within first 48 hours of birth, temporary conductive conditions may contribute to high referral rates (Doyle et al., 2000; Allen et al., 2005).

Many studies have reported the prevalence of conductive hearing loss due to transient middle ear dysfunction such as otitis media with effusion (OME) to be higher than sensorineural (SN) hearing loss (Doyle et al., 2000; Keefe et al., 2000; Boone et al., 2005; Hunter et al., 2010; Silverman, 2010; Aithal et al., 2012). For example, in a retrospective study of 76 infants, Boone et al. (2005) attributed 64.5% of failures in UNHS to OME. In another follow up study of 211 infants referred for diagnostic assessment following AABR screening, 32% had a conductive loss due to middle ear dysfunction (Aithal et al., 2012). Similarly, Boudewyns et al. (2011) reported 55.3%, Holster et al. (2009) reported 18.4%, and Doyle et al. (2004) reported 58% of failures due to OME. These studies also noted that OME is an important cause of transient hearing loss during the first months of life (Doyle et al., 2004; Holster et al., 2009; Boudewyns et al., 2011). These studies also highlighted that infants who failed in UNHS due to OME are at increased risk for later development of chronic otitis media.

Despite the high referral rates due to middle ear dysfunction, there is presently no single validated objective tool that can be used at the time of screening to assess middle ear function (Keefe et al., 2000). Assessment of middle ear function is not currently part of the Joint Committee on Infant Hearing (JCIH, 2007) guidelines for UNHS programs. Middle ear assessment is only recommended for newborns as part of the diagnostic assessment. Hence, differential diagnosis of transient conductive loss and permanent SN loss can only be made during follow up diagnostic assessments which are expensive and time consuming. For this reason, development of screening tools to assess middle ear function at the time of newborn screening is recommended (Gravel et al., 2005). Such tools would assist in streamlining the management strategies for the respective types of hearing loss, facilitating prioritisation of neonates for follow-up appointments and reducing parental anxiety.

The only definitive tests for the presence of OME are myringotomy or imaging studies such as computerised axial tomography (CT) scanning. However, neither of these techniques is practical or ethical under screening conditions. Otoscopy is not reliable in newborns due to difficulties in observing changes in colour, mobility, reflexive reaction to lights and translucency of the ear drum (Paradise, 1980; Ruah et al., 1991; Rhodes et al., 1999) and it cannot be relied upon in the diagnosis of OME in newborns (Shurin et al., 1976; Roberts et al., 1992). For example, Shurin et al. (1976) noted that five out of 10 ears diagnosed with OME by otoscopy were found to have normal or dry ear on tympanocentesis. Similarly, Doyle et al. (1997) observed that half of the 9% of ears diagnosed as having OME based on reduced ear drum mobility on pneumatic otoscopy, passed the AABR screening test and about one-third passed the transient evoked OAE (TEOAE) screening test, indicating that the use of pneumatic otoscopy in young infants can result in incorrect diagnosis.

Previous attempts using conventional 226-Hz tympanometry to diagnose middle ear dysfunction in young infants (≤ 6 months of age) have been unsuccessful (Paradise, 1976; Keefe & Levi, 1996; Rhodes et al., 1999; Purdy & Williams, 2002; Baldwin, 2006).The use of high frequency tympanometry (HFT) with 1000 Hz probe tone has been reported to be more successful (Purdy & Williams, 2002; Kei et al., 2003; Margolis et al., 2003; Baldwin, 2006; Swanepoel et al., 2007). The HFT is currently recommended by JCIH (2007) for diagnostic testing after UNHS referrals. The sensitivity and specificity of HFT in detecting conductive conditions in newborns using distortion product OAE (DPOAE) as a reference standard are reported to be 50% and 91%, respectively (Margolis et al., 2003). While Swanepoel et al (2007) reported a similar test performance result of sensitivity of 57% and specificity of 95% for HFT against a DPOAE reference standard, Baldwin (2006) reported a sensitivity of 99% and specificity of 89% using automated brainstem response outcomes as the reference standard for older infants with a mean age of 10 weeks. However, Baldwin's findings might not apply for neonates as the youngest infant in her study was two weeks old. Although HFT is recommended for use with young infants (Kei et al., 2003; Margolis et al., 2003; Baldwin, 2006; Alaerts et al., 2007), there are no universally agreed methods for interpreting results (Kei & Mazlan, 2012). It is also shown that introducing negative and positive air pressure distends an infant ear canal and modifies the middle ear characteristics (Holte et al., 1990). It also violates the underlying assumptions of tympanometry in infants (Margolis & Shanks, 1990; Kei & Zhao, 2012). For instance, on pressurization as in tympanometry, the diameter of ear canal increases by an average of 18.3% under positive

pressure or decreases by 28.2% under negative pressure compared to its original value (Holte et al., 1990). Furthermore, ear canal volume changes from 27 to 75% over a range of ± 300 daPa in newborns (Qi et al., 2006). In addition to these limitations, measurement of peak compensated static admittance (Ypc) for the negative tail method introduces artifactual spikes and danger of collapsing the ear canal with negative ear canal pressure (Kei et al., 2007; Aithal et al., 2016; Hunter & Blankenship, 2017). It is also reported that the positive tail method overestimates ear canal volume and is less sensitive to middle ear dysfunction in young infants (Hunter & Blankenship, 2017). Given such limitations of HFT, there is a need to introduce alternative techniques to identify middle ear dysfunction in young infants.

Wideband absorbance (WBA) is an emerging technology which can assess middle ear function in young infants and could be a useful tool in UNHS (Keefe et al., 2003; Sanford et al., 2009; Hunter et al., 2010; Hunter et al., 2015). However, it is not yet recommended by JCIH (2007) for diagnostic testing of infants. Recently, Aithal et al. (2015a) attempted to use WBA technology to identify conductive conditions in newborns. The authors evaluated the test performance of WBA against commonly used reference standards using the receiver operating characteristics (ROC) curve analysis. Their results showed that optimal test performance of the WBA, as indicated by the area under the ROC curve (AROC), reached 0.78 when compared against test battery reference standards (Aithal et al., 2015a). Sanford et al (2009) and Hunter et al (2010) reported better performance of WBA than HFT in predicting conductive conditions in newborn screening whereas other studies have reported that both HFT and WBA are excellent measures to identify transient conductive hearing loss in young infants (Prieve et al., 2013b). Sanford et al. (2009) reported the highest AROC of 0.86 (95% CI: 0.80 – 0.89) for WBA and 0.75 (95% CI: 0.68 – 0.80) for HFT with DPOAE screening as the reference standard on day one. On day two, they reported an AROC of 0.67 (95%CI: 0.45 – 0.83) for WBA and 0.54 (95% CI: 0.36 – 0.71) for HFT. Similarly, Hunter et al. (2010) reported that wideband reflectance (WBR) produced much better prediction of DPOAE status in newborns than HFT and reported an AROC of 0.72 for HFT, 0.82 for WBR at 1 kHz and 0.90 for WBR at 2 kHz. Although WBA performed better than HFT, both measures were proven to be effective in detecting conductive conditions in neonates (Sangster, 2011).

An alternative measure of outer and middle ear function, the sweep frequency impedance (SFI) technology, has been found to be more accurate than tympanometry in

diagnosing middle ear diseases in adults (Wada & Kobayashi, 1990; Wada et al., 1998). However, the application of SFI to detect conductive conditions in young infants has not been investigated until recently (Murakoshi et al., 2012; Murakoshi et al., 2013; Aithal et al., 2014; Aithal et al., 2015b; Aithal et al., 2016). The SFI method, developed in the 1990s, measures the resonance frequency (RF) and mobility (ΔSPL) of the outer and middle ear system at different static pressures in the ear canal as well as tympanometric peak pressure (TPP, the pressure at which the SPL attains maximum value) (Wada & Kobayashi, 1990; Murakoshi et al., 2012; Zhao & Wang, 2012). Although the technology appears to measure impedance, it actually measures the sound pressure level (SPL) in the ear canal while a sweeping tone is presented under various static pressure levels in the ear canal. From these SPL curves, the dynamic behaviour of the outer and middle ear can be described in a graph showing the sound pressure level (in dB SPL) against frequencies from 100 to 2200 Hz at various static pressures applied to the ear canal. From the SPL curves, the RF and ΔSPL can be measured.

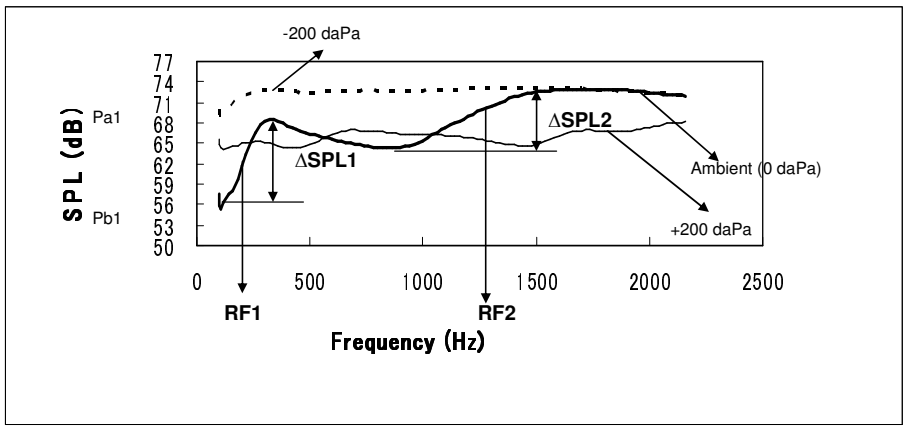
The dynamic behaviour of the outer and middle ear system, as measured using an SFI meter, of a healthy newborn is shown in Figure 6.1(a). The greatest variations of sound pressure level (ΔSPL) occur at median frequencies of RF1 and RF2 along the SPL curve (at ambient pressure of 0 daPa) which are considered as the RF of the ear canal and middle ear, respectively (Wada et al., 1995; Murakoshi et al., 2013). In comparison, the SFI results for a newborn, who failed in HFT and TEOAE, reveal only one variation at RF1 (Aithal et al., 2016) (Figure 6.1(b)).

Previous studies have demonstrated that the SPL curves obtained from neonates have shown two variations i.e., low frequency (210-420 Hz) and high frequency (830-1500 Hz) regions (Murakoshi et al., 2013; Aithal et al., 2014). This suggests that there are two vibrating elements in the neonatal auditory system, possibly due to external and middle ear components. The second variation in the higher frequency region (RF2) was similar to that of adult middle ear resonance frequency reported in the Wada et al. (1998) study.

The first variation in the low-frequency region (RF1) was thought to be caused by an element other than the middle ear. It was considered to be associated with the movement of the external ear canal wall as Young's modulus of which is estimated to be 0.36 times as much as that of adults, i.e., 36-364 kPa (Saunders et al., 1983; Qi et al., 2006). The resonance movements of the neonatal ear canal have been reported to be lower than 450 Hz

(Keefe et al., 1993). In addition, studies based on a neonatal external ear canal physical model using agarose gel and a numerical model using finite element method suggest that the external ear canal wall exhibits intrinsic oscillatory behaviour at around 300 Hz (Murakoshi et al., 2013; Hamanishi et al., 2015). Hence, the first variation of the SPL curve obtained from neonates in the low-frequency region (RF1) is considered to be related to the resonance of the neonatal external ear canal wall movements (Murakoshi et al., 2013; Hamanishi et al., 2015; Wada et al., 2016).

(a)



(b)

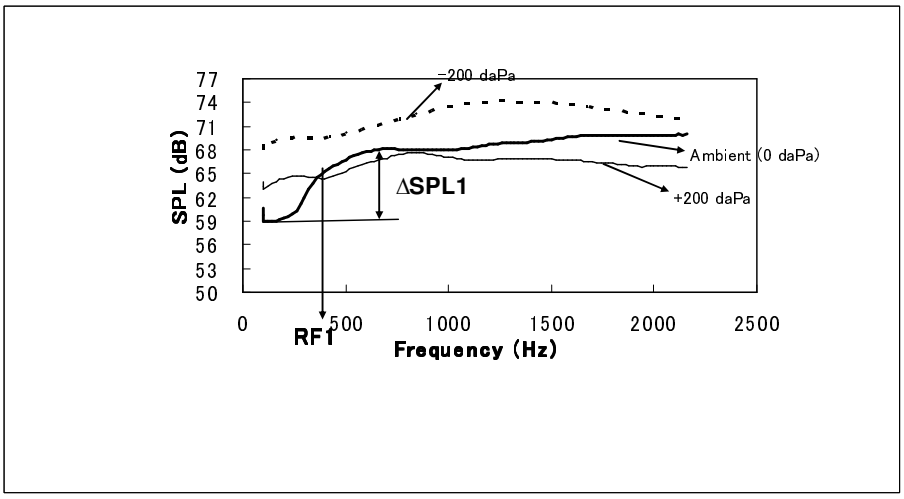


Figure 6.1. Typical SFI results obtained (a) from a healthy one-day-old newborn who passed the test battery. The SPL curve at ambient pressure shows two inflexions in sound pressure (RF1 and RF2). The greatest variations of SPL (ΔSPL) occurs at median frequencies of RF1 and RF2 which is considered as the RF of the ear canal and middle ear, respectively, (b) from a one-day-old newborn who did not pass test battery. Note: The SFI curve at ambient pressure shows only one inflexion (RF1). The static ear canal pressure (daPa) applied were +200, 0 (ambient), and -200 daPa. RF = resonance frequency.

As illustrated in Figure 6.1(b), Murakoshi et al (2012) and Aithal et al (2016) noted that the second resonance (RF2) disappeared in newborns with middle ear dysfunction while the first resonance (RF1) was not affected. These results indicated that middle ear dysfunction altered the dynamic behaviour of the middle ear to such an extent that the second resonance could not be detected using the SFI meter. SFI measures have potential advantages over HFT in assessing infants. First, since pressurisation of the ear canal is not required when SFI measures are obtained at ambient pressure, distortion or collapse of the ear canal wall is not a concern. Second, measurements are made over a wide frequency range from 100 to 2200 Hz, rather than at a single frequency, and finally, SFI can provide additional information such as resonance frequency and mobility of the middle ear which may assist in diagnosing conductive conditions in young infants (Murakoshi et al., 2012; Aithal et al., 2016). While the SFI shows promising results in identifying dysfunction of the outer and middle ear in newborns, the test performance of SFI in determining the middle ear status of newborns has not been evaluated against any reference standard. It is of utmost importance to investigate the predictive accuracy of SFI in comparison to other reference standards before the SFI can be used as a mass screening tool for identifying conductive conditions in newborns. The aim of this study was, therefore, to evaluate the test performance of SFI to predict the middle ear status in newborns against clinical reference standards including 4 single tests (AABR, HFT, DPOAE and TEOAE) and their combinations (HFT+DPOAE, HFT+TEOAE, DPOAE+TEOAE, DPOAE+AABR and TEOAE+AABR).

6.4 Methods

This study was approved by the Human Research Ethical Committee of Townsville Hospital and Health Service, and the University of Queensland Behavioural and Social Sciences Ethical Review Committee (Appendix 1). Parents provided written consent for newborns to be included in the study (Appendix2). All infants were born at full term, with normal birth weight and no medical complications or risk factors for hearing loss.

6.4.1 Participants

In total, 188 (98 males, 90 females) healthy neonates were recruited for the present study. All measurements were performed in a quiet room in the maternity unit where noise level was below 40 dBA. Nursing staff performed the AABR screen on both ears of all

neonates as part of a state mandated universal newborn hearing screening. AABR screening was performed using an ALGO3 newborn hearing screener (Natus Medical Inc. Pleasanton, CA). Clicks were presented at 35 dB nHL to both ears simultaneously during testing. A pass or refer result for each ear was automatically recorded by the equipment.

6.4.2 Procedure

An experienced audiologist conducted HFT, DPOAE, TEOAE and SFI tests on the neonates with the accessible ear tested first, followed by the second ear if possible. HFT was performed using a Madsen Otoflex 100 acoustic immittance device (GN Otometrics, Taastrup, Denmark) with a 1000-Hz probe tone of 75 dB SPL delivered to the ear (Mazlan et al., 2009). Admittance (Ya) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec. Pass criteria were a single positively peaked tympanogram with middle ear pressure between 50 and -150 daPa and peak compensated static admittance Ypc (+200 daPa tail to peak) of at least 0.2 mmho (Mazlan et al., 2009)

DPOAE testing was performed using a Scout sport (Biologic Navigator Plus, Mundelein, IL) system. DPOAEs were obtained in response to stimulation by pairs of primary tones. The f2/f1 frequency ratio was 1.2 for each primary pair. The level of f1 was 65 dB SPL and f2 was 55 dB SPL. The 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 SPL at 2, 3, 4 and 6 kHz (Sanford et al., 2009; Merchant et al., 2010).

TEOAE testing was conducted using same device as mentioned above. Wideband clicks of 80 µs duration were delivered to the ear at 80 dB pkSPL. Emissions were measured at 1, 1.5, 2, 3 and 4 kHz. The pass criteria included reproducibility of at least 70% and a signal-to-noise ratio (SNR) of at least 3 dB at 2, 3, and 4 kHz (Kei et al., 2003; Vander Werff et al., 2007).

The SFI test was performed using a new SFI unit developed for testing neonates (Murakoshi et al., 2013; Aithal et al., 2014). The SFI unit and its calibration have been described in detail by Murakoshi et al. (2013) and Aithal et al. (2014). A brief description of the SFI unit is provided here. Figure 6.2 shows a block diagram of the SFI unit which consisted of a personal computer, an analog-to-digital/digital-to-analog (AD/DA) converter, a

probe system, a stepping motor, an air pump, a pressure sensor, and a pressure relief valve. The probe consisted of 3 tubes: the first tube to apply static pressure (Ps) to the ear canal, the second tube to deliver sound to the external ear canal via an earphone, and the third tube to measure sound pressure in the external ear canal using a microphone. A specially designed cuff suitable for testing neonates was attached to the tip of the probe to obtain a hermetic seal during testing. This new SFI unit was controlled using LabView under MS WINDOWS. The SFI unit also performs HFT on infants as part of the test procedures.

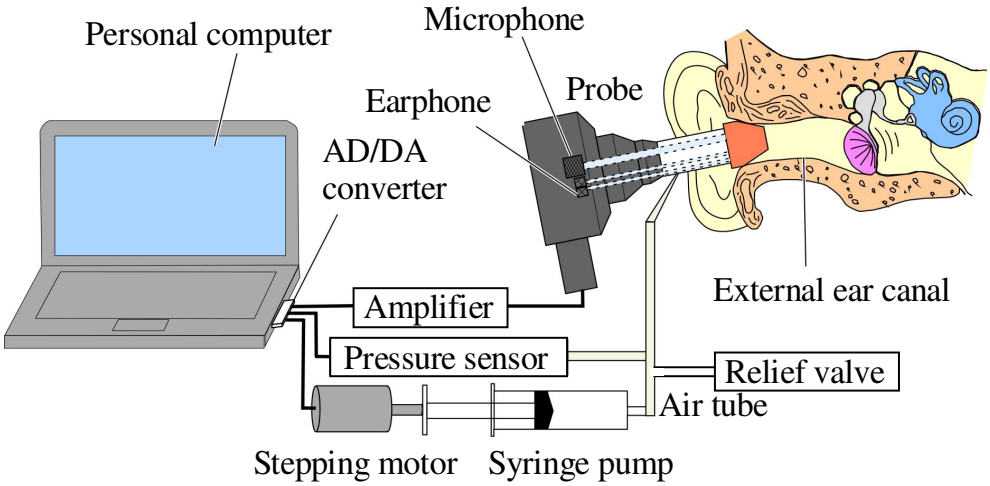


Figure 6.2. Block diagram of SFI meter used to test newborns in this study. The SFI meter consists of a personal computer, an AD/DA converter, a probe system, a stepping motor, a syringe pump, a pressure sensor and a relief valve. This new SFI meter is controlled using LabView (Wada et al. 2016).

During the SFI test, the sound pressure level in the ear canal was measured as the frequency of the pure tone stimulus was swept from 100 to 2200 Hz while the external auditory canal static pressure (Ps) was held constant at +200 daPa. This measurement was repeated with Ps reduced in 50 daPa steps down to -200 daPa. The entire SFI procedure was automated, taking less than one minute to complete the test in each ear. A sweeping probe tone was delivered to the ear at 75 dB SPL to reduce the risk of eliciting an acoustic stapedial reflex. While the SFI results measured at multiple static pressures provide a comprehensive view of the acoustic-mechanical properties of the outer and middle ear, Murakoshi et al (2013) and Aithal et al. (2014) found that measurements made at ambient pressure (0 daPa) can provide adequate clinical information about the status of the outer and middle ear. For the

purpose of the present study, only measurements performed at ambient pressure were included in the analyses.

The pass/refer criteria for the SFI measures in the present study were based on normative data developed by Aithal et al. (2014) using the same SFI unit. Aithal et al. (2014) noted two regions of resonance in newborns, with the mean RF1 for the first resonance occurring at 287 Hz (90% range: 209-420 Hz) and the mean RF2 for the second resonance occurring at 1236 Hz (90% range: 830-1518 Hz). The first and second resonances refer to the resonances of the ear canal and middle ear, respectively. The authors' subsequent study (Aithal et al., 2016) showed that absence of the second resonance was associated middle ear dysfunction in newborns. The presence of the second resonance with RF2 value of between 830 and 1518 Hz was considered as a pass (indicating normal middle ear function).

6.4.3 Reference standards and pass/refer classification

At present, there is no complete agreement on which "reference" standard should be used to determine the test performance of diagnostic tests for the detection of disorders of the sound conduction pathways in newborns. Researchers have used both DPOAE (Margolis et al., 2003; Sanford et al., 2009; Hunter et al., 2010) and TEOAE (Kei et al., 2003; Vander Werff et al., 2007; Shahnaz, 2008) to determine the status of the middle ear such that absent or low level OAEs are suggestive of middle ear disorders in the absence of a sensorineural hearing loss. Although Norton et al. (2000b) found no difference in the performance of TEOAE and DPOAE to detect hearing loss in newborns, the inclusion of both TEOAE and DPOAE as reference standards in the present study would be useful because the mechanism involved in generating OAEs by the two procedures are different. Furthermore, the two procedures demonstrate different susceptibility to background and biological noise, resulting in different test outcomes (Rhoades et al., 1998; Shi et al., 2000; Norton et al., 2000a; Norton et al., 2000b; Norton et al., 2000c).

The present study used 9 reference standards (4 single tests and 5 test batteries) for determining the test performance of SFI. Table 6.1 shows the 9 reference standards adopted in this study. While a single test such as DPOAE is useful in identifying conductive disorders in newborns, it also has limitations which compromise its predictive accuracy. For this reason, test battery reference standards involving a combination of tests were used in the present study. From a clinical perspective, newborns who passed a battery of tests involving

HFT and DPOAE were more likely to have a normal sound conduction pathway (outer and middle ear) than those who passed DPOAE or HFT only (Aithal et al., 2013; Aithal et al., 2015a). In case of test battery reference standards, a strict test protocol was used (Keefe et al., 2003). With this protocol, the ear with a pass in all the tests in any given test battery was included in the 'pass' group for that reference standard. For instance, with the HFT+DPOAE reference standard, only ears with a pass in both HFT and DPOAE tests were included in the pass group for that test battery. Likewise, ears with a refer in each test of the test battery were included in the 'refer' group for that reference standard. For example, with HFT+TEOAE reference standard, only the ears with a refer in both HFT and TEOAE tests were included in the 'refer' group for that reference standard. While this strict test protocol provides clear separation between the pass and refer groups (Keefe et al., 2003; Aithal et al., 2015a), it excludes ears that have passed one test, but failed in the other test. If the ears that failed either of the tests are omitted, the test performance (sensitivity and specificity values) would be inflated. However, if the ears that failed in either the OAE or HFT test were classified in the "refer" category, the sensitivity and specificity values would be deflated. The present study, having adopted a strict test protocol, acknowledged this as a limitation of the study.

Table 6.1 shows the number of ears that passed or referred in each of the 9 reference standards adopted in this study. For example, for the DPOAE reference standard, 40 ears referred and 223 ears passed out of 263 ears. When DPOAE was combined with HFT in a test battery (HFT+DPOAE) reference standard, the number of ears referred was reduced to 21 out of a total of 220 ears.

6.4.4 Data analysis:

All analyses were performed using the using the SPSS software (version 22). In the present study, RF2 values between 830 and 1518 were considered as a pass (normal) while RF2 values greater than 1518 or absence were considered as a refer (abnormal). This normative range was based on the results of a previous study (Aithal et al., 2014). The test performance of SFI was determined in terms of the sensitivity, specificity and AROC.

Table 6.1. Reference standard adopted in this study showing number of ears that passed or referred in each group

Reference Standard	Ears passed	Ears referred	Total
SFI	222	41	263
AABR	254	9	263
HFT	218	45	263
DPOAE	223	40	263
TEOAE	201	62	263
HFT+DPOAE	199	21	220
HFT+TEOAE	184	28	212
DPOAE+TEOAE	198	37	235
AABR+DPOAE	222	8	230
AABR+TEOAE	200	8	208

SFI: Sweep frequency impedance

AABR: automated auditory brainstem response

HFT: high frequency tympanometry

DPOAE: distortion product otoacoustic emission

TEOAE: transient evoked otoacoustic emissions

The test performance of SFI could also be determined using the Likelihood ratio (LR) analysis. The LR of a test refers to improvement in the likelihood of making a correct diagnosis or identifying a condition. Positive likelihood ratio (LR+) refers to improvement of likelihood of correctly identifying the presence of a condition, whereas negative likelihood ratio (LR-) refers to improvement of likelihood of correctly identifying the absence of a condition. In general, a test with LR+ greater than 10 and LR- less than 0.1 is considered to be an effective test.

6.5 Results

Table 6.2 shows the details of newborns included in the study. All newborns had uneventful birth history with no risk factors for hearing loss (JCIH, 2007). The study included 263 ears (133 right and 130 left) from 188 healthy newborns (98 males and 90 females). Table 6.2 also shows the mean and standard deviation for gestational age (in weeks), birth weight (in grams), and age of testing (in hours) for 188 newborns. In order to maximise the

data, each ear was considered independent of each other because each ear could have provided a different outcome (pass or refer). Although this maximised the available data, the authors are aware that there may be correlations in measurements between the right and left ear.

Table 6.2. Details of infants included in this study.
N= 188 infants. Total ears = 263 (R: 133; L:130); SD = standard deviation

	Male	Female	Total
No of neonates	98	90	188
Right ear	29	29	58
Left ear	27	28	55
Bilateral	42	33	75
Gest age (weeks)			
Mean	39.4	39.3	39.3
SD	1.29	1.1	1.2
Birth weight (grms)			
Mean	3537.9	3386.6	3465.4
SD	414.8	490.7	457.8
Age at time of testing (hours)			
Mean	44.4	47	45.6
SD	22.8	19	21

An AROC was computed to determine the test performance of SFI against 4 single test and 5 test battery reference standards. The results for the 9 reference standards adopted in this study are shown in Table 6.3. Among the 4 single test reference standards, AROC was the greatest for HFT (0.82; 95% CI: 0.74 - 0.89) and smallest for AABR (0.61; 95% CI: 0.41-0.81). In comparison, among the 5 test battery reference standards, AROC was the greatest for the HFT+DPOAE reference standard (0.87; 95% CI: 0.78 - 0.96). AROC was the smallest for the AABR+TEOAE reference standard (0.66; CI: 0.45-0.88). AROC was significantly greater than 0.5 for HFT, DPOAE and TEOAE single test and for HFT+DPOAE, HFT+TEOAE, and DPOAE +TEOAE test battery reference standards, as determined using

the statistical procedure described by Hanley and McNeil (1982) ($p<0.05$). These results indicate that the ability of SFI in identifying conductive conditions was inferior when the AABR, AABR+DPOAE and AABR+TEOAE reference standards were used because the AROCs were not significantly different from 0.5.

Table 6.3. Test performance of SFI against different reference standards as determined by AROC

Reference Standard	AROC	95% CI	Std. error	Significance
AABR	0.61	0.41 - 0.81	0.10	0.26
HFT	0.82	0.74 - 0.89	0.04	0.00*
DPOAE	0.69	0.59 - 0.79	0.05	0.00*
TEOAE	0.62	0.54 - 0.71	0.04	0.00*
HFT+DPOAE	0.87	0.78 - 0.96	0.05	0.00*
HFT+TEOAE	0.82	0.72 - 0.91	0.05	0.00*
DPOAE+TEOAE	0.69	0.59 - 0.79	0.05	0.00*
AABR+DPOAE	0.67	0.45 - 0.88	0.11	0.12
AABR+TEOAE	0.66	0.45 - 0.88	0.11	0.12

Null hypothesis: true area = 0.5; *: indication of statistical significance (Significantly different from 0.5 with $p<0.005$)

AABR: automated auditory brainstem response

HFT: high frequency tympanometry

DPOAE: distortion product otoacoustic emission

TEOAE: transient evoked otoacoustic emissions

Table 6.3 also shows the 95% confidence interval (CI) for AROC when SFI outcomes were compared with the outcomes of the 9 reference standards. The CIs for the AABR, AABR+DPOAE, and AABR+TEOAE reference standards were broad and inferior to other 6 reference standards. Although the CIs for DPOAE, TEOAE, and DPOAE+TEOAE were better, they were still inferior to that for the HFT, HFT+DPOAE, and HFT+TEOAE reference standards. Table 6.4 shows the sensitivity, specificity, LR+ and LR- for SFI against both single and test battery reference standards. Among the 4 single reference standards, SFI had the highest sensitivity and specificity of 76% and 88%, respectively, against HFT. Among the test battery reference standards, SFI had the highest sensitivity and specificity of 86% and 88%, respectively against the HFT+DPOAE (Table 6.4). For the single reference

standards, the LR+ reached a highest value of 6 for HFT (or LR- = 0.3) and lowest value of 2 for AABR (or LR- = 0.7). For the test battery reference standards, the LR+ reached a highest value of 7 for HFT+DPOAE (or LR- = 0.2) and lowest value of 3 for DPOAE+TEOAE, AABR+DPOAE and AABR+TEOAE (or LR- = 0.6).

Table 6.4.Table showing the sensitivity, specificity, and likelihood ratios of SFI for different reference standards.

Reference Standard	Sensitivity (Estimate in %)	Specificity (Estimate in %)	LR+	LR-
AABR	44	78	2	0.7
HFT	76	88	6	0.3
DPOAE	55	83	3	0.5
TEOAE	42	83	3	0.7
HFT+DPOAE	86	88	7	0.2
HFT+TEOAE	75	88	6	0.3
DPOAE+TEOAE	54	83	3	0.6
AABR+DPOAE	50	82	3	0.6
AABR+TEOAE	50	83	3	0.6

AABR: automated auditory brainstem response

HFT: high frequency tympanometry

DPOAE: distortion product otoacoustic emission

TEOAE: Transient Evoked Otoacoustic Emissions

LR+: Positive likelihood ratio

LR -: Negative likelihood ratio

As mentioned above, CIs for HFT, HFT+DPOAE and HFT+TEOAE were similar and superior to other reference standards (Table 6.3). In order to determine whether the AROCs of HFT, HFT+DPOAE and HFT+TEOAE reference standards were significantly different from each other, a statistical test as described by (Hanley & McNeil, 1982) was applied using an online vassarstats test (http://vassarstats.net/roc_comp.html). The results showed no significant difference in AROC between the HFT and HFT+ DPOAE (p=0.44), HFT and HFT+TEOAE (p=1.00), and HFT+DPOAE and HFT+TEOAE reference standards (p=0.48).

6.6 Discussion

The present study evaluated the test performance of SFI in terms of its ability to identify conductive conditions in newborns against various single test and test battery reference standards. As a single non-invasive gold standard does not exist for diagnosing conductive conditions in neonates, nine audiologic test reference standards were used for comparison with SFI in this study.

The present study showed that the test performance of SFI against the HFT reference standard was higher than that against any of the AABR, DPOAE and TEOAE reference standards as revealed by their respective AROC values (Table 6.3). These results imply that the test outcomes of SFI compare more favourably with that of the HFT test than with the AABR, DPOAE and TEOAE tests, indicating the ability of SFI to detect conductive conditions in neonates. Since HFT could identify conductive conditions in newborns with high accuracy (Baldwin, 2006) and that HFT's performance was as good as that of wideband reflectance (Prieve et al., 2013a,b), it is reasonable to infer that SFI identifies conductive disorders in newborns with reasonably good accuracy. Further analysis comparing SFI results with HFT outcomes revealed a sensitivity of 76% and specificity of 88% (Table 6.4). The LR+ and LR- were 6 and 0.3, respectively. The larger the LR+ or lower the LR- (close to zero), the better is the performance of a test. In general LR+ greater than 10 (or LR- less than 0.1) is considered as a very useful test (large effect), 5 to 10 (or LR- 0.1 to 0.2) as a useful test (moderate effect), 2 to 4.9 (or LR- 0.21 to 0.5) as a somewhat useful test (small effect), and 1 to 1.9 (or LR- 0.51 to 1) as a rarely useful test (very small effect) (Ebell 2016). Although LR did not reach the preferred values of LR+ >10 or LR - <0.1, these results suggest that SFI is a diagnostically useful test (Ebell, 2016).

In contrast, the test performance of SFI against the AABR reference standard was the lowest among the 4 single test reference standards. The AROC for AABR was 0.61 which was not significantly different from 0.5, indicating that SFI could not predict the outcomes of AABR more than chance. Studies have shown that AABR is not sensitive to slight /mild conductive hearing losses (Stapells, 2000; 2011; Aithal et al., 2012). Furthermore, a refer result in AABR may indicate a significant sensorineural hearing loss which will not be detected by the SFI.

The test performance of SFI against TEOAE and DPOAE was low (Table 6.3 and 6.4) but significantly greater than 0.5, indicating that the test performance of SFI against these

reference standards was better than chance in identifying conductive conditions in newborns (Table 3). One of the reasons for TEOAE and DPOAE not being ideal reference standards is that they are intended to detect auditory disorders up to the inner ear. TEOAE and DPOAE tests are also limited in their ability to accurately assess conductive hearing loss below 2 kHz where conductive disorders obliterate reverse transmission of emissions at these frequencies. Furthermore, OAE test results are affected by environmental and physiologic noises which may produce a refer outcome in a normally hearing child. Like AABR, a refer result in an OAE test may indicate a significant sensorineural hearing loss which will not be detected by the SFI.

The test performance of SFI against the HFT+DPOAE test battery reference standard was high with an AROC of 0.87, which was significantly better than that of AABR, DPOAE, TEOAE and DPOAE+TEOAE (Table 6.3). This superior test performance was expected because a combination of tests may often detect a disease condition with higher accuracy than that of an individual test alone (Baughman et al., 2008; Naaktgeboren et al., 2013). For instance, inclusion of HFT in a test battery with DPOAE (HFT+DPOAE) increased sensitivity from 55% to 86%. In general, the accuracy of SFI was superior with the inclusion of HFT in the test battery reference standard and inferior with the inclusion of AABR in the test battery reference standard. The positive and negative likelihood ratios were 7 and 0.2, respectively. Although LR did not reach the preferred values of LR+ >10 or LR - <0.1, these result suggest that SFI can be considered as diagnostically useful test with a moderate effect and that the likelihood of making a correct diagnosis was enhanced when a more strict reference standard was used.

Although it is not possible to directly compare the present investigation with other studies which have used wideband acoustic immittance measures, it is noted that the test performance of SFI against the DPOAE reference standard was better than that previously reported. For example, Sanford et al. (2009) reported an AROC of 0.86 (95% CI: 0.80-0.89) for WBA and an AROC of 0.75 (95% CI: 0.68 – 0.80) for HFT against the DPOAE reference standard on day one of screening. However, on day two of screening, the AROC was reduced to 0.67 (95% CI: 0.45 – 0.83) for WBA and 0.54 (95% CI: 0.36-0.71) for HFT. In comparison, the present study showed an AROC of 0.69 (95% CI: 0.59 – 0.79) for SFI against the DPOAE reference standard which is better than that obtained by the Sanford et al. (2009) study on day two of screening. The results of the present study are also better than the

highest AROC of 0.67 for WBA at 1.25 kHz against a DPOAE reference standard obtained by Aithal et al. (2015a). However, Hunter et al. (2010) reported more enhanced values than those of the present study with an AROC of 0.82 and 0.90 for WBR at 1 and 2 kHz, respectively. The age of testing could be the one of the reason for these differences as majority of infants were tested within the first 24 to 48 hours after birth in Hunter et al. (2010) study which is earlier than present study.

The sensitivity and specificity of SFI against the DPOAE reference standard in identifying conductive conditions, as shown in the present study, were 55% and 83%, respectively (Table 6.4). These results are consistent with the findings of Margolis et al. (2003) who reported a sensitivity of 50% and specificity of 91% for HFT against a DPOAE reference standard. Swanepoel et al. (2007) reported slightly better sensitivity of 57% and specificity of 95% for HFT against a DPOAE reference standard. In summary, the performance of SFI against DPOAE was comparable to that of HFT against DPOAE in identifying conductive conditions in newborns.

6.6.1 Clinical application

The high test performance of SFI against the test battery (HFT+DPOAE) and single test (HFT) reference standards suggests that SFI is a valid measure of the function of the middle ear in newborns. Hence, SFI may be employed in UNHS programs as an adjunct test to the AABR screen. The clinical information provided by the SFI test may be useful for prioritising newborns for further diagnostic testing. A neonate who failed the SFI but passed the AABR test will receive follow-up assessments to determine if the conductive condition has been resolved. A neonate who passed the SFI, but failed in the AABR test would require further diagnostic assessments to determine the degree and nature of the hearing deficit. In the worst scenario when a neonate has failed in both SFI and AABR tests, a referral for diagnostic audiology assessment along with referral to an otolaryngologist is recommended because of the possibility of middle ear dysfunction along with sensorineural hearing loss. In addition, the test performance of SFI justifies its application as a diagnostic test in UNHS follow-up testing. However, further research is required to evaluate the predictive accuracy of SFI in other age groups and ears with different conductive disorders.

6.6.2 Limitations

One of the limitations of the present study was the use of a strict protocol for determination of pass or refer status when a test battery reference standard was used. This strict protocol had excluded the ears that "passed" in one test but "referred" in the other test within the test battery reference standard. The adoption of this strict protocol did not only reduce the sample size, consequently reducing the power of the statistical analyses, but it also would have inflated the AROC, sensitivity and specificity values for the SFI against the test battery reference standards.

Although it was relatively easy to test well-settled newborns, the SFI test took approximately 1 minute to conduct in each ear. Since all tests had to be completed using different equipment in the same session, the removal and re-insertion of probes would sometimes disturb the neonate. Calming the neonate often increased the test duration. If the neonate became unsettled, the chance of having incomplete data collection increased. Further research is recommended using equipment that allows all tests to be done using a single probe. Further improvement in SFI instrumentation is needed to speed up the test if it is to be used as an assessment tool in newborn screening and diagnostics.

The outcome of the study could have also been influenced by the pass and refer criteria of some tests. For instance, the pass criterion for HFT was a single positive peak with positive peak admittance (Ya) of ≥0.2 mmho, while double or multiple peaks were considered as a refer in this study. Similarly, the TEOAE criterion of at least a 3 dB SNR in three frequency regions (2, 3, and 4 kHz) and DPOAE criterion of minimum SNR of 6 dB and DPOAE amplitude greater than -6 dB SPL in at least three out of four f2 frequencies (2, 3, 4, and 6 kHz) might not give optimal results. Furthermore, these pass criteria did not adequately assess frequencies below 2 kHz where the impact of middle ear disorders on audition is more prominent.

The test performance of SFI may vary depending on the time of screening during the postnatal period. The mean age of screening newborns in the present study was 45.6 hours, but with a substantial standard deviation of 21 hours. Studies have shown that transient conductive conditions due to the presence of vernix and/or mesenchyme in the outer and middle ear may occur within the first 48 hours of birth (Doyle et al., 2000; Allen et al., 2005; Sanford et al., 2009). The transient conductive conditions would affect the referral rates of neonates in all screening tests which, in turn, affect the test performance of SFI. Hence, it is

very important to consider the time of screening after delivery as a contributing factor when comparing the test performance of different protocols.

There is lack of a "true" gold standard for testing middle ear function in newborns. Although test battery reference standards were used to determine normal outer/middle ear sound conduction function, a pass in all of these tests cannot definitively rule out slight outer/middle ear dysfunction in newborns (Aithal et al., 2012).

6.7 Conclusions

The test performance of SFI was compared against 4 single test and 5 test battery reference standards in this study. The test performance of SFI against HFT with an AROC of 0.82 and against HFT+DPOAE with an AROC of 0.87 indicates that SFI can accurately identify conductive conditions in newborns. Hence, SFI test can be used for both screening and diagnostic assessments in newborns.

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6.9 References

Aithal S., Aithal V., Kei J. & Driscoll C. 2012. Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia. *J. Am. Acad. Audiol.*, 23, 1-13.

Aithal S., Kei J., Driscoll C. & Khan A. 2013. Normative wideband reflectance measures in healthy neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 77, 29-35.

Aithal S., Kei J., Driscoll C., Khan A. & Swanston A. 2015a. Wideband Absorbance Outcomes in Newborns: a comparison with high-frequency tympanometry, automated brainstem response, and transient evoked and distortion product otoacoustic emissions. *Ear. Hear.*, 36, e237-e250.

Aithal V., Kei J., Driscoll C., Murakoshi M. & Wada H. 2016. Effects of ear canal static pressure on the dynamic behaviour of outer and middle ear in newborns. *Int. J. Pediatr. Otorhinolaryngol.*, 82, 64-72.

Aithal V., Kei J., Driscoll C., Swanston A., Murakoshi M., et al. 2015b. Sweep frequency impedance measures in Australian Aboriginal and Caucasian neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 79, 1024-1029.

Aithal V., Kei J., Driscoll C., Swanston A., Roberts K., et al. 2014. Normative sweep frequency impedance measures in healthy neonates. *J. Am. Acad. Audiol.*, 25, 343-354.

Alaerts J., Luts H. & Wouters J. 2007. Evaluation of middle ear function in young children: clinical guidelines for the use of 226- and 1,000- Hz tympanometry. *Otol. Neurotol.*, 28, 727-732.

Allen J.B., Jeng P.S. & Levitt H. 2005. Evaluation of human middle ear function via an acoustic power assessment. *J. Rehabil.Res.Dev*, 42 (suppl.2), 63-77.

Baldwin M. 2006. Choice of probe tone and classification of trace patterns in tympanometry undertaken in early infancy. *Int. J. Audiol.*, 45, 417-427.

Balkany T.J., Berman S.A., Simmons M.A. & Jafek B.W. 1978. Middle ear effusions in neonates. *The Laryngoscope*, 88, 398-405.

Baughman A.L., Bisgard K.M., Cortese M., M., Thompson W.W., Sanden G.N., et al. 2008. Utility of composite reference standards and latent class analysis in evaluating the clinical accuracy of diagnostic tests for pertussis. *Clin. Vaccine. Immunol*, 15, 106-114.

Boone R.T., Bower C.M. & Martin P.F. 2005. Failed newborn hearing screens as presentation of otitis media with effusion in the newborn population. *Int. J. Pediatr. Otorhinolaryngol*, 69, 393-397.

Boudewyns A., Declau F., Van den Ende J., Van Kerschaver E., Dirckz S., et al. 2011. Otitis media with effusion: an underestimated cause of hearing loss in infants. *Otol. Neurotol,*32, 799-807.

Buch N.H. & Jorgensen M.B. 1964. Embroynic connective tissue in the tympanic cavity of the fetus and newborn. *Acta. Otolaryngica.*, 58, 111-126.

Clemens C.J. & Davis A. 2001. Minimizing false positive in universal newborn hearing screening: A simple solution. *Pediatrics*, 107, 1-3.

Clemens C.J., Davis A. & Bailey A.R. 2000. The False-positive in universal newborn hearing screening. *Pediatrics*, 106, 1-5.

Derkay C.S., Bluestone C.D., Thompson A.E., Stephenson J.S. & Kardtzke D. 1988. Otitis media in the pediatric intensive care unit; A prospective study of its incidence, prevalence, bacteriology, etiology and treatment. *Ann. Otol. Rhinol. Laryngol.*, 97 (suppl) 133, 56-57.

Doyle K.J., Kong Y.Y., Strobel K., Dallaire P. & Ray R.M. 2004. Neonatal middle ear effusin predicts chronic otitis media with effusion. *Otol. Neurotol.*, 25, 318 - 322

Doyle K.J., Rodgers P., Fujikawa S. & Newman E. 2000. External and middle ear effects on infant hearing screening test results. *Otolaryngol. Head. Neck. Surg.*, 122, 477-481.

Doyle K.J., Burggaaff B., Fujikawa S., Kim J. & Macarthur C.J. 1997. Neonatal hearing screening with otoscopy, auditory brainstem response and otoacoustic emissions. *Otolaryngol. Head. Neck. Surg* 116, 597-603.

Ebell M.H. 2016. Evidence-based practice for health professions: a free online course: chapter 4 - diagnostic tests-ROC curves. The University of Georgia, College of Public Health.

Gravel J.S., White K.R., Johnson J.L., Widen J.E., Vohr B.R., et al. 2005. A multisite study to examine the efficacy of the otoacoustic emission/automated auditory brainstem response newborn hearing screening protocol: recommendations for policy, practice and research. *Am. J. Audiol.*, 14, S217-S228.

Hamanishi S., Murakoshi M., Steele C., Wada H. & Puria S. 2015. A hybrid ear canal and middle ear model approach for neonates. *Assoc. Res. Otolaryngol.*, Abs. , PS 145.

Hanley J.A. & McNeil B.J. 1982. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*., 143, 29-36.

Holster I.L., Hoeve L.J., Wieringa M.H., Willis-Lorier R.M.S. & de Gier H.H.W. 2009. Evaluation of hearing loss after failed neonatal hearing screening. *J. Pediatr*., 155, 646-650.

Holte L., Cavanaugh R.M. & Margolis R.H. 1990. Ear canal wall mobility and tympanometric shape in young infants. *J. Pediatr.*, 117, 77-80.

Hunter L.L. & Blankenship C.M. 2017. Middle ear measurement. *In:* A. Tharpe, M. & R. Seewald (eds.) *Comprehensive handbook of pediatric audiology*. San Diego: Plural Publishing.

Hunter L.L., Feeney M.P., Miller J.A.L., Jeng P.S. & Bohning S. 2010. Wideband reflectance in newborns: normative regions and relationship to hearing-screening results. *Ear. Hear.*, 31, 599-610.

Hunter L.L., Keefe D.H., Feeney M.P., Fitzpatrick D.F. & Lin L. 2015. Longitudinal development of wideband reflectance tympanometry in normal and at-risk infants. *Hear. Research.*, In press.

JCIH 2007. Joint Committee on infant Hearing. Year 2007 Position statement: Principles and guidelines for early hearing detection and intervention programs. *Pediatrics.,* 120, 898-921.

Keefe D.H., Bulen J.C., Arehart K.H. & Burns E.M. 1993. Ear canal impedance and reflection coefficient in human infants and adults. *J. Acoust. Soc. Am.*, 94, 2617-2638.

Keefe D.H. & Feeney M.P. 2009. Principles of acoustic imittance and acoustic transfer functions. *In:* J. Katz, R.F. Burkhard & L. Medwetsky (eds.) *Handbook of clinical audiology*. Baltimore: Lippincott, Williams and Wilkins.

Keefe D.H., Folsom R.C., Gorga M.P., Vohr B.R., Bulen J.C., et al. 2000. Identification of neonatal hearing impairment: ear-canal measurements of acoustic admittance and reflectance in neonates. *Ear. Hear.*, 21, 443-461.

Keefe D.H., Gorga M.P., Neely S.T., Zhao F. & Vohr B. 2003. Ear-canal acoustic admittance and reflectance measurements in human neonates. II. Predictions of middle-ear dysfunction and sensorineural hearing loss. *J. Acoust. Soc. Am.*, 113, 407-422.

Keefe D.H. & Levi E. 1996. Maturation of the middle and external ears: acoustic power-based responses and reflectance tympanometry. *Ear. Hear.*, 17, 361-373.

Kei J., Allison-Levick J., Dockray J., Harrys R., Kirkegard C., et al. 2003. High-frequency (1000 Hz) tympanometry in normal neonates. *J. Am. Acad. Audiol.*, 14, 20-28.

Kei J. & Mazlan R. 2012. High-Frequency (1000 Hz) Tympanometry: Clinical applications. *In:* J. Kei & F. Zhao (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 39-67.

Kei J., Mazlan R., Hickson L., Gavranich J. & Linning R. 2007. Measuring middle ear admittance in new borns using 1000 Hz tympanometry: A comparison of methodologies. *J. Am. Acad. Audiol.*, 18, 739-748.

Kei J. & Zhao F. 2012. The challenge of assessing middle ear function in young infants: Summary and future directions. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 163-177.

Kok M.R., vanZanten G.A. & Brocaar M.P. 1992. Growth of evoked otoacoustic emissions during the first days postpartum. *Audiology*., 31, 140-149.

Margolis R.H., Bass-Ringdahl S., Hanks W.D., Holte L. & Zapala D.A. 2003. Tympanometry in newborn infants- 1 kHz norms. *J. Am. Acad. Audiol.*, 14, 383-392.

Margolis R.H. & Shanks J.E. 1990. Tympanometry: Basic principles and clinical applicatons. . *In:* W.F. Rintelmann (ed.) *Hearing Assessment*. Austin, TX: Pro-Ed, pp. 179-246.

Mason J.A. & Herrmann K.R. 1998. Universal infant hearing screening by automated auditory brainstem response measurement. *Pediatrics*., 101, 221-228.

Mazlan R., Kei J. & Hickson L. 2009. Test-retest reliability of the acoustic stapedial test in healthy neonates. *Ear. Hear.*, 30, 295-301.

Mehl A.L. & Thomson A.E. 2002. The Colorado newborn hearing screening project, 1992-1999: On the threshold of effective population-based universal newborn hearing screening. *Pediatrics*., 109;e7, 1-8.

Mehl A.L. & Thomson V. 1998. Newborn hearing screening; The great Omission. *Pediatrics*., 101;e4, 1-10.

Merchant G.R., Horton N.J. & Voss S.E. 2010. Normative reflectance and transmittance measurements on healthy newborn and 1-month-old infnats. *Ear. Hear.*, 31, 1-9.

Murakoshi M., Yoshida N., Sugaya M., Ogawa Y., Hamanishi S., et al. 2013. Dynamic characteristics of the middle ear in neonates. *Int. J. Pediatr. Otorhinolaryngol.*, 77, 504-512.

Murakoshi M., Zhao F. & Wada H. 2012. Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 107-129.

Naaktgeboren C.A., Bertens L.C., van Smeden M., de Groot J.A., Moons K.G., et al. 2013. Value of composite reference standards in diagnostic research. *BMJ*, 347, f5605.

Norton S.J., Gorga M.P., Widen J.E., Folsom R.C., Sininger Y., et al. 2000a. Identification of neonatal hearing impairment: summary and recommendation. *Ear. Hear.*, 21, 529-535.

Norton S.J., Gorga M.P., Widen J.E., Folsom R.C., Sininger Y., et al. 2000b. Identification of neonatal hearing impairment: Evaluation of transient evoked otoacoustic emission, distortion prodcut otoacoustic emission and auditory brainstem response test performance. *Ear. Hear.*, 21, 508-528.

Norton S.J., Gorga M.P., Widen J.E., Folsom R.C., Sininger Y., et al. 2000c. Identification of neonatal hearing impairment: A multi-center investigation. *Ear. Hear.*, 21, 348-356.

Paradise J.L. 1976. Pediatrician's view of middle ear effusion: More questions than answers. *Ann. Otol. Rhinol. Laryngol.*, 25 Suppl 85, 20-24.

Paradise J.L. 1980. Otitis media in infants and children. *Pediatrics*., 65, 917-943.

Paradise J.L. 1981. Otits media during early life: how hzardous to development? A critical review of the evidence. *Pediatrics.*, 68, 869-873.

Prieve B.A., Feeney M.P., Stenfelt S. & Shahnaz N. 2013a. Prediction of conductive hearing loss using wideband acoustic immittance. *Ear. Hear.*, 34, 54s-59s.

Prieve B.A., Vander Werff K., Preston J.L. & Georgantas L. 2013b. Identificaton of conductive hearing loss in young infants using tympanometry and wideband reflectance. *Ear. Hear.*, 34, 168-178.

Purdy S.C. & Williams S.M. 2002. High frequency tympanometry: A valid and reliable immittance test protocol for young infants. *NZ. Audiolog. Soc. Bull.*, 10, 9-24.

Qi L., Liu H., Lutfy J., Funnell W.R. & Daniel S.J. 2006. A nonlinear finite-element model of the newborn ear canal. *J. Acoust. Soc. Am.*, 120, 3789-3798.

Rhoades K., McPherson B., Smyth V., Kei J. & Balglioni A. 1998. Effects of background noise on click-evoked otoacoustic emissions. *Ear. Hear.*, 19, 450-462.

Rhodes M.C., Margolis R.H., Hirsch J.E. & Napp A.P. 1999. Hearing screening in the newborn intensive care nursery: comparison of methods. *Otolaryngol. Head. Neck. Surg.*, 120, 799-808.

Rosenfeld R.M., Culpepper L., Doyle K.J., Grundfast K., Hoberman A., et al. 2004. Clinical practice guideline: otitis media with effusion. *Otolaryngol. Head. Neck. Surg.*, 130 (Suppl), S95-S118.

Ruah C.B., Schachern P.A., Zelterman D., Paperella M. & Yoon T. 1991. Age related morphologic changes in the human tympanic membrane. A light and electron microscopic study. *Arch. Otolaryngol. Head. Neck. Surg.*, 117, 627-634.

Sanford C.A., Keefe D.H., Liu Y.-W., Fitzpatrick D.F., McCreery R.W., et al. 2009. Sound-conduction effects on Distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry. *Ear. Hear.*, 30, 635-652.

Sangster L. 2011. Critical review: Can wideband energy reflectance be used in newborn hearing screening to detect transient middle ear dysfunction and to interpret screening results?: University of Western Ontario, pp. 1-6.

Saunders J.C., Kaltenback J.A. & Relkin E.M. 1983. The structural and functional development of the outer and middle ear. *In:* R. Romand & M.R. Romand (eds.) *Development of Auditory and Vestibular systems*. New York: Academic Press, pp. 3-25.

Shahnaz N. 2008. Wideband reflectance in neonatal intensive care units. *J. Am. Acad. Audiol.*, 19, 419-429.

Shi S., Kei J., Murdoch B., McPherson B., Smyth V., et al. 2000. Pediatric hearing screening in the community: a comparison of outcomes from transient evoked and distortion product otoacoustic emission measures. *Scand. Audiol.*, 29, 83-92.

Shurin P.A., Pelton S.I. & Klein J.O. 1976. Otitis meida in the newborn infant. *Ann. Otol. Rhinol. Laryngol.*, 85 (Suppl 250, 216-222.

Silverman C.A. 2010. Multifrequency tympanometry in infants and children *Audiologyonline.*, pp. 1-18.www.audiologyonline.com/articles/multifrequency-tympanometry-in-infants-and-852

Stapells D.R. 2000. Threshold estimation by the tone evoked auditory brainstem response: A literature metanalysis. *J. Speech-Lang. Pathol and Audiol.*, 24, 74-83.

Stapells D.R. 2011. Frequency-specific threshold assessment in young infants using the transient ABR and the brainstem ASSR. *In:* R. Seewald & A. Tharpe, M. (eds.) *Comprehensive handbook of pediatric audiology*. San Diego: Plural, pp. 409-448.

Swanepoel D.W., Werner S., Hugo R., Louw B., Owen R., et al. 2007. High freqeuncy immitance for neonates: A normative study. *Acta. Otolaryngol.*, 127, 49-56.

Vander Werff K.R., Prieve B.A. & Georgantas L.M. 2007. Test-retest reliability of wideband reflectance measures in infants under screening and diagnostic test conditions. *Ear. Hear*, 28, 669-681.

Vohr B.R., Carty L.M., Moore P.E. & Letourneau K. 1998. The Rhode Island hearing assessment program: experience with statewide hearing screening (1993-1996). *J. Pediatr.*, 133, 353-357.

Wada H. & Kobayashi T. 1990. Dynamical behavior of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus. *J. Acoust. Soc. Am.*, 87, 237-245.

Wada H., Koike T. & Kobayashi T. 1998. Clinical applicability of the sweep frequency measuring appartus for diagnosis of middle ear diseases. *Ear. Hear.*, 19, 240-249.

Wada H., Murakoshi M., Aithal V., Kei J., Hamanishi S., et al. 2016. Dynamic characteristic of the outer and middle ears in neonates. *33rd World congress of Audiology*. Vancouver.

Wada H., Ohyama K., Kobayashi T., Koike T. & Noguchi S. 1995. Effect of middle ear on otoacoustic emissions. *Audiology.*, 34, 161-176.

Zhao F. & Wang J. 2012. Assessing middle ear function in humans using multifrequency tympanometry: An overview. *In:* J. Kei. & F. Zhao. (eds.) *Assessing middle ear function in infants*. San Diego: Plural, pp. 87-106.

Chapter 7: Discussion and Conclusions

7.1 Introduction

This chapter revisits the rationale and aims of the study, as described in Chapter 1, and discusses the main findings presented in previous chapters. Conclusions with clinical implications of the studies included in this thesis are presented. Recommendations and suggestions for future research are also discussed in this chapter.

7.2. Rationale for the study (revisited)

The present research was designed to study the applications of SFI in Australian neonates and infants. Although UNHS programs have been introduced in all states and territories in Australia, there are no published studies which report false positive referrals (due to conductive pathology) in the Australian context, except for a single study which investigated conductive hearing loss and middle ear pathology in young infants referred through a UNHS program (S. Aithal et al., 2012).

For successful clinical applications of SFI technology with neonates and young infants, it is important to develop clinical norms to distinguish normal status from abnormal conductive conditions. While many researchers have used DPOAE or TEOAE as a reference standard for determining normal middle ear function in infants and children (Driscoll et al., 2001; L. L. Hunter et al., 2010; Kei et al., 2003; Merchant, Horton, & Voss, 2010; C.A. Sanford & Brockett, 2014; C. A. Sanford & Feeney, 2008), it has been noted that OAEs may not be an ideal "gold standard" because they do not identify sub-clinical middle ear dysfunctions in infants (Driscoll et al., 2001; L. L. Hunter et al., 2010; Kemp, Ryan, & Bray, 1990; C. A. Sanford et al., 2009). However, it was suggested that a test battery reference standard may provide greater assurance of normal middle ear function than a single test (Mazlan & Kei, 2012). The study, described in Chapter 2, investigated the feasibility of testing neonates using a new SFI unit designed for testing neonates and young infants and provided normative SFI data using test battery reference standards which included a combination of AABR, TEOAE, and HFT tests.

The outer and middle ear system in newborns is not mature, and its dynamic behaviour is altered when an external air pressure is applied to the ear canal (Holte et al., 1990; Qi et al., 2008; Qi et al., 2006). On pressurisation, the dynamic behaviour of the outer and middle ear of newborns changes, depending on the pressure applied to the ear canal. The study, described in Chapter 3, illustrated the effects of ear canal static pressure on the dynamic behaviour of the outer and middle ear in newborns with and without conductive conditions. The study also provided useful clinical information on collapsing ear canals under substantial negative pressures.

The outer and middle ear system of infants undergoes rapid developmental changes from birth to 6 months of age, which influence the transmission of sound through the middle ear. This means that SFI results will change as a function of age. Hence, age specific normative SFI data are needed. The study, described in Chapter 4, investigated the developmental characteristics of SFI measures in healthy infants from birth to 6 months, as well as providing normative data for different age groups.

Australian Aboriginal children have one of the highest rates of OM that starts early in life and remains throughout their childhood. Despite high prevalence of OM in this group of children, there is limited research regarding the status of the middle ear at birth. To date, there have been no studies that used SFI to evaluate outer and middle ear function in Australian Aboriginal neonates. The study, described in Chapter 5, compared SFI results between Australian Aboriginal and Caucasian neonates with and without conductive conditions.

Evaluating the test performance of SFI is a challenging task. The definitive tests for the presence of OME are myringotomy and imaging studies such as CT scanning which are neither practical nor ethical for use with neonates who are suspected of having a conductive condition. Even though ABR (AC and BC threshold assessment) is considered as a surrogate gold standard for identifying middle ear dysfunction in infants, this method is time consuming and not practical during the neonatal period. Hence, most studies circumvent this issue by using OAE as the “gold standard” because it is commonly used as a screening tool in UNHS programs (Sangster, 2011). As mentioned earlier, OAEs may not accurately identify minor middle ear dysfunction and may not serve as an ideal reference standard. Hence, a variety of reference standards including single tests and multiple tests were employed in this

study. The study, described in Chapter 6, evaluated the predictive accuracy of SFI to determine the status of the middle ear in newborns against nine different reference standards.

7.3 Aims of the thesis (restated)

Given the above rationales for the study, the aims of this research were:

(1) To investigate the feasibility of testing neonates using the SFI technique, describe the dynamic behaviour of the outer and middle ear in healthy neonates who passed a battery of tests including AABR, TEOAE and HFT, and establish normative SFI data for resonance frequency (RF) and mobility of the outer and middle ear in terms of changes in sound pressure level (Δ SPL in dB) (see Chapter 2).

(2) To measure the effect of ear canal static pressure on the dynamic behaviour of the outer and middle ear in healthy newborns (see Chapter 3).

(3) To conduct a cross-sectional study to determine the developmental characteristics of SFI measures on a sample of normal young infants aged 0, 1, 2, 4 and 6 months (see Chapter 4).

(4) To compare SFI measures obtained from healthy newborn Australian Aboriginal infants with those obtained from Caucasian infants (see Chapter 5).

(5) To evaluate the predictive accuracy of SFI in terms of its ability to identify conductive conditions in neonates when compared with 9 different reference standards consisting of single tests and composite test batteries including HFT, AABR, TEOAE and DPOAE (see Chapter 6).

7.4 Hypothesis of the study (restated)

The present study contained four null hypotheses (H_0) to be tested. They are:

H_0 1: There is no significant difference in mean values of SFI measures (RF and Δ SPL) of the outer and middle ear when the static ear canal pressure is changed from +200 to -200 daPa in newborns with and without conductive conditions.

H_0 2: There is no significant age effect on SFI measures obtained from infants aged 0 (birth) to 6 months.

H_0 3: There is no significant difference in SFI measures between Australian Aboriginal and Caucasian neonates.

H_0 4: There is no significant difference in the predictive accuracy of SFI between single tests and test battery reference standards.

7.5 Discussion of the main findings

7.5.1 Normative SFI measures in healthy neonates

As described in Chapter 2, the aims of this study were to investigate the feasibility of testing neonates using the new SFI unit, describe the dynamics of the outer and middle ear in healthy Australian neonates and establish normative SFI data. A prospective sample of 100 neonates (58 males and 42 females), with mean gestational age of 39.3 wk (SD = 1.3 wk) who passed all three tests, namely AABR, TEOAE and HFT (1000 Hz), were included in this study.

The study demonstrated that SFI is a feasible test of outer and middle ear function in neonates. The study also showed that the low resonance frequency (RF1) is possibly related to the resonance movements of the outer ear canal wall and the higher resonance frequency (RF2) is related to the resonance of the middle ear. The study clearly demonstrated that it is feasible to measure middle ear as well as ear canal resonance movements using the SFI technique, which is a distinct advantage over other middle ear tests. The study also used a new robust "reference standard" requiring all neonates to pass a test battery without using an invasive procedure such as myringotomy to confirm middle ear status.

The normative SFI data revealed two distinct resonance regions, indicating functional differences in the acoustical properties of the outer ear (RF1 & ΔSPL1) and middle ear (RF2 & ΔSPL2). The high RF (RF2) with mean value of 1236 Hz (SD = 200 Hz; 90% range = 830–1518 Hz) was approximately equal to four times that of the low RF (RF1) with mean value of 287 Hz (SD = 69 Hz; 90% range = 209 - 420 Hz). The Δ SPL1 at RF1 with mean value of 8.2 dB (SD = 3.1 dB; 90% range = 3.4 - 13 dB) was greater than that at RF2 with mean value of 5 dB (SD = 2 dB; 90% range = 1.5 - 8.1 dB). The study did not show any significant differences or interactions between genders and ears. The normative data developed in this study will be useful in evaluating outer and middle ear function in neonates.

7.5.2 Effects of ear canal static pressure on dynamic behaviour of outer and middle ear in newborns

As described in Chapter 3, this study investigated the effect of ear canal static pressure on the dynamic behaviour of the outer and middle ear in newborns with and without a conductive condition using the SFI technology. A test battery consisting of AABR, TEOAE, and HFT was performed on 122 ears of 86 healthy newborns and 10 ears of 10 newborns with a conductive condition. When the pressure applied to the ear canal was varied from 200 to -200 daPa, the dynamic behaviour of the outer and middle ear was evaluated in terms of the SPL in the ear canal, RF and displacement (Δ SPL).

The study showed that the application of positive or negative pressure to the ear canal increased RF1 and RF2, but reduced Δ SPL1 and Δ SPL2, in comparison to those obtained under ambient pressure (0 daPa). These findings indicate that the outer and middle ear system becomes stiffer under pressurised conditions than under ambient pressure. Furthermore, the dynamic behaviour of the outer and middle ear under positive pressures was distinctively different to that under negative pressures. The positive static pressures in the ear canal resulted in lower SPL in the ear canal, whereas negative static pressures resulted in higher SPL than that at ambient pressure. In addition, more than 90% of ears showed evidence of collapse when static ear canal pressure was decreased to -200 daPa. The results of static pressures on the dynamic behaviour of the middle ear in ears with conductive condition showed a distinctive pattern, with evidence of resonance in the low frequency region (RF1), but no resonance in the high frequency region (i.e., RF2 could not be measured). Consequently, the null hypothesis $H_0$1, which stated that there is no significant difference in mean values of SFI measures (RF and Δ SPL) of the outer and middle ear when static ear canal pressure is changed from +200 to -200 daPa in newborns with and without conductive conditions, was rejected. However, in the case of the 10 newborns with a conductive condition, RF2 and ΔSPL2 could not be determined because the resonance of the middle ear was absent.

The study showed that the SFI measure in newborns is useful for differentiating healthy ears from ears with a conductive condition. The dynamic behaviour of the outer and middle ear under positive pressures was distinctively different to that under negative pressures. There was evidence of collapsed ear canals when a substantial negative pressure (e.g., -200 daPa) was applied to the ear canal. The study deduced that measuring of peak compensated static admittance using the negative tail compensation method might produce unreliable results in the presence of a collapsing ear canal in neonates.

7.5.3 Developmental characteristics of SFI measures from birth to 6 months

This study, as described in Chapter 4, investigated developmental characteristics of SFI in infants in their first 6 months of life. The study group included 117 healthy ears from 83 infants. SFI was measured in 30 ears of 24 neonates (birth), 28 ears of 16 one-month-olds, 18 ears of 13 two-months-olds, 22 ears of 17 four-month-olds, and 19 ears of 13 six-month-olds.

The study showed that mean RF1 increased from 279 Hz at birth to 545 Hz at 4 months of age. At the same time, the mean mobility of the outer ear (Δ SPL1) decreased from 7.9 dB at birth to 3.7 dB at 4 months. In addition to this, RF1 could be measured in only 73% of ears at 4 months of age and none (0%) at 6 months of age. These data illustrate a fast maturation process of the outer ear during the first 6 months. However, no such trend of increasing or decreasing resonance of middle ear (RF2) and mobility (Δ SPL2) with age was noted. The mean RF2 for different age groups from birth to 6 months of age fell between 1174 Hz to 1395 Hz, which is within the previously reported normative range of 830 – 1520 Hz for newborns (V. Aithal et al., 2014). Therefore, the null hypothesis $H_0 2$ which stated that there is no significant age effect on SFI measures obtained from infants aged 0 (birth) to 6 months was rejected.

SFI data obtained at ambient pressure (0 daPa) and TPP were also compared across the age groups. Significant differences were noted only between ambient and TPP for RF1 and ΔSPL1 at birth, and for ΔSPL1 at 1 month of age. No significant differences were noted between ambient and TPP for RF2 and mobility ΔSPL2 from birth to 6 months of age.

In summary, the present study showed clear evidence of maturation of the outer ear whereby RF1 increased and Δ SPL1 decreased with age, indicating increasing stiffness of the outer ear with age which could be used as a developmental marker for ear canal development. In contrast, SFI data did not show significant changes in RF2 and ΔSPL2. The normative data developed in this study for the different age groups may serve as a reference for clinical evaluation of the function of the conductive pathway (outer and middle ear) in young infants.

7.5.4 SFI measures in Australian Aboriginal and Caucasian neonates

Although the prevalence of middle ear dysfunction in Australian Aboriginal children is high, there is very limited research on the acoustic-mechanical properties of their outer and middle ear status at birth. Thus, there is a need to investigate the outer and middle ear status of Australian Aboriginal neonates using new technologies of middle ear assessment such as SFI. This study, as described in Chapter 5, compared the acoustic-mechanical properties of the outer and middle ear between Australian Aboriginal and Caucasian neonates using SFI measures.

Data was collected from 40 ears of 24 Australian Aboriginal neonates (16 males, 8 females) with mean gestational age of 39.6 wk (SD = 1.3 wk) and 160 ears of 119 Caucasian neonates (57 males, 62 females) with mean gestational age of 39.3 wk (SD=1.3 wk) who passed a test battery that included AABR, DPOAE, and HFT. The present study revealed that Aboriginal neonates had significantly lower mean resonance frequency for the outer ear (mean RF1=264.9 Hz, SD=58.6 Hz) and middle ear (mean RF2=1144 Hz, SD=228.8 Hz) than that of Caucasian neonates (mean RF1=295.3 Hz, SD=78.4 Hz and mean RF2=1241.8 Hz, SD=216.6 Hz). Interestingly, no significant differences in the mobility of the outer ear (Δ SPL1) and middle ear (Δ SPL2) between the two groups were found, despite a tendency for Aboriginal neonates to have lower ΔSPL1 and ΔSPL2 values than their Caucasian counterparts. Moreover, middle ear resonance was absent in 22.5% of Aboriginal neonate ears, but present in all Caucasian ears. This finding suggests that Australian Aboriginal neonates may have subtle conductive conditions that were detected by SFI, but not by the test battery. Hence, the null hypothesis ($H_0$3), which stated that there is no significant difference in SFI measures between Australian Aboriginal and Caucasian neonates, was rejected.

Since SFI is a sensitive test for middle ear function, addition of SFI to the test battery in UNHS programs could improve detection of middle ear dysfunction in this population. Although it is possible that anatomical and physiological characteristics of the two ethnic groups could have resulted in different SFI findings, the factors contributing to the differences remain undetermined and require further investigation.

7.5.5 Predictive accuracy of SFI technology

Before the SFI can be used as a clinical measure, it is important to evaluate its predictive accuracy in the detection of conductive conditions in neonates. In this study, as described in Chapter 6, the test performance of SFI was assessed against nine different

reference standards that included 4 single tests (AABR, HFT, TEOAE and DPOAE) and 5 test batteries (HFT+DPOAE, HFT+TEOAE, DPOAE+TEOAE, DPOAE+AABR and TEOAE+AABR) using receiver operating characteristic (ROC) analysis and traditional test performance measures such as sensitivity and specificity.

Among the four single test reference standards, AROC was the greatest for HFT (0.82; 95% CI: 0.74 - 0.89) and smallest for AABR (0.61; 95% CI: 0.41-0.81). In comparison, among the five test battery reference standards, AROC was the greatest for the HFT+DPOAE reference standard (0.87; 95% CI: 0.78 - 0.96). AROC was the smallest for the AABR+TEOAE reference standard (0.66; CI: 0.45-0.88). AROC was significantly greater than 0.5 for HFT, DPOAE and TEOAE single test and for HFT+DPOAE, HFT+TEOAE, and DPOAE +TEOAE test battery reference standards, as determined using the statistical procedure described by Hanley and McNeil (1982) ($p<0.05$). These results indicate that the ability of SFI to identify conductive conditions was inferior when the AABR, AABR+DPOAE and AABR+TEOAE reference standards were used because the AROCs were not significantly different from 0.5. Therefore, the null hypothesis $H_0 4$, which stated that there is no significant difference in the predictive accuracy of SFI between single tests and test battery reference standards, was rejected. The results also revealed that the test performance of SFI against the test battery reference standard of HFT+DPOAE and single reference standard of HFT were high with an AROC of 0.87 and 0.82, respectively. Although the HFT+DPOAE test battery reference standard performed better than the HFT reference standard in predicting middle ear conductive conditions in neonates, the difference in AROC was not significant. Further analysis revealed that the highest sensitivity and specificity for SFI (86% and 88%, respectively) was obtained when compared with the reference standard of HFT+DPOAE. Among the four single reference standards, SFI had the highest sensitivity and specificity (76% and 88%, respectively), when compared against the HFT reference standard. The positive and negative likelihood ratios were 7 and 0.2, respectively.

The low test performance of SFI against the single test reference standards indicates that a single test such as AABR, HFT or OAEs may not accurately detect conductive conditions in neonates. Possible reasons for this include: (1) the test is not sensitive to slight/mild conductive conditions, (2) the test is susceptible to physiological and/or environmental noises, (3) the test can only provide limited clinical information about the

properties of the conductive pathway, (4) the pass criteria set for the test were not optimal, and (5) AABR and OAEs are not direct tests of outer and middle ear function.

While accepting the limitations of single test reference standards, the use of a test battery consisting of HFT and OAE enhanced the predictive accuracy of SFI compared to single test reference standards. This indicates that SFI could provide a more effective, alternative method than a combination of tests for detecting conductive conditions in neonates. This finding is promising as SFI can be used for both screening and diagnostic assessments in neonates.

7.6 Implication for clinical practice

7.6.1 Application of SFI as an adjunct screening test during UNHS

Based on the results of several studies conducted as part of this thesis, SFI appears to be a valid tool for evaluation of middle ear status and can be used as an adjunct test in UNHS programs. This proposition is based on the following research findings: (1) SFI had high test performance against test battery (HFT+DPOAE) and single test (HFT) reference standards in identifying dysfunctions of the conductive system in newborns (Chapter 6); (2) SFI could identify the conductive status of the outer and middle ear at the same time, and easily separate ears with middle ear dysfunction in the presence of normal outer ear condition in neonates (Chapter 3); (3) SFI could easily identify negative static ear canal pressure at which ear canal collapses in neonates (Chapter 3); (4) SFI could be used as a developmental marker of outer ear maturation (Chapter 4), and; (5) SFI could identify Australian Aboriginal infants with subtle middle ear conditions that could not be identified by a battery of tests (Chapter 5).

The high predictive accuracy of SFI technology in identifying conductive conditions in newborns clearly suggests that SFI could be used as an adjunct test to reduce false positive referrals and prioritise infants referred for diagnostic evaluation through UNHS programs. By incorporating a set of pass and refer criteria for SFI, an automated response (pass or refer) could be displayed similar to that used in AABR or automated OAE devices. As these results do not need to be interpreted by screening staff, the SFI unit could be used by screening personnel in conjunction with other screening tests used in UNHS programs.

In Australia, all states and territories, except South Australia (SA), use AABR screening for their UNHS programs. ACT adopts a three-stage AABR screening protocol whereas other states adopt a two-stage AABR screening protocol. SA adopts a three-stage automated OAE and AABR protocol. Despite the increased false positive outcomes due to middle ear dysfunction, there is presently no single validated objective tool that has been adopted for use with newborns to assess middle ear function (Keefe et al., 2000). Assessment of middle ear function is not currently part of the Joint Committee on Infant Hearing (JCIH, 2007) guidelines for UNHS programs. Presently, middle ear assessment is only recommended for newborns as part of diagnostic assessments. Hence, differential diagnosis of transient conductive loss and permanent SN loss can only be made during follow up diagnostic assessments which are expensive and time consuming. For this reason, development of screening tools to assess middle ear function at the time of newborn screening has been suggested (Gravel et al., 2005).

SFI technology could be easily adapted to UNHS programs to reduce false positive responses due to sound conduction (outer and middle ear) dysfunction. According to this proposed model, infants who do not pass their first AABR/OAE screening would be rescreened as usual before their discharge from the hospital or screening clinic. If the infant does not pass the second screening (one or both ears), SFI screening should be considered. It is proposed that neonates who obtain a refer result during the second AABR/OAE screening, but pass SFI screening (normal outer and middle ear status), would be at risk of sensorineural hearing loss. Hence, they should be immediately referred to a paediatric audiologist for diagnostic assessments within two weeks or as per the state and territory UNHS protocol. However, neonates with a refer result in both SFI and second screening would receive a third AABR/OAE screening within four to six week time. If they receive a refer result in the third screening, further diagnostic audiologic evaluations would be recommended. Use of SFI screening as an adjunct screening test following second AABR/OAE screen could easily determine if a conductive condition does exist in the presence of a refer result. This strategy would assist to streamline management for neonates with respective types of hearing loss, facilitate prioritisation for follow-up for diagnostic appointments and reduce parental anxiety.

Introduction of SFI as an adjunct screening test following the second screen would determine whether diagnostic audiology tests are required (in case of pass result) or third screen with AABR/OAE in case of a refer outcome. Many studies have reported reduced

false positive referral rates when a three-stage protocol is used for UNHS programs and given support to such protocols because the reduced referral rate can decrease expensive diagnostic assessments. The false positive referral rate can be reduced further if SFI is included in the test battery as an adjunct screening test because only neonates with hearing loss would be referred for diagnostic assessment. During the second screening, only neonates with risk of SN hearing loss would be referred, while during the third screening, neonates with persisting conductive and mixed hearing loss would be identified. In this way, the majority of neonates whose hearing returns to normal with the resolution of conductive conditions would not be referred for diagnostic evaluation.

This three-stage protocol with the inclusion of SFI would also assist in the prioritisation of neonates at risk of SN loss for diagnostic assessment. This would assist in early diagnosis of SN loss and reduce parental anxiety, as diagnostic evaluation is recommended once a pass is obtained in SFI screening immediately after the second AABR/OAE referral. In this way, health resources could be better used for those neonates who passed SFI following the second AABR/OAE referral, to ensure that they receive timely diagnostic assessment and early intervention without loss to follow up. In addition to this, the use of SFI as an adjunct screening tool could provide additional information about the middle ear status and, therefore, assist in the diagnosis of ambiguous results obtained during diagnostic evaluation, and assist in the cross-checking of results. This would also reduce unnecessary costs for rural and remote families who need to travel long distances with young infants for diagnostic assessments.

7.6.2 Application of SFI during diagnostic evaluation of neonates and young infants

7.6.2.1 SFI during diagnostic evaluation of neonates

SFI has the potential to detect conductive conditions in neonates with high accuracy given the high test performance of SFI when evaluated against test battery (HFT+DPOAE) and single test (HFT) reference standards (Chapter 6). Hence, SFI could be used as a single clinical test with test performance which is as good as the test battery reference standards. The SFI test showed a sensitivity of 86% and specificity of 88% against the test battery reference standard (HFT+DPOAE).

The present study established normative SFI data for outer (RF1) and middle ear (RF2) resonance frequency and mobility of outer (ΔSPL1) and middle ear (ΔSPL2) in terms of changes in sound pressure level using test battery reference standards of AABR, HFT and TEOAE (Chapter 2). The study provided mean, SD, 90% range and median data as well as explained the dynamic behaviour of the outer and middle ear. These normative SFI measures could be used clinically to determine the status of the sound conductive mechanism in neonates. Neonates with SFI values falling within this normative range are regarded as having an efficient conductive pathway, while those with SFI values falling outside the range would require further investigations.

The results of the present study also showed that there are two regions of resonance, one at low frequency (RF1) and another at high frequency (RF2), in newborns, thus indicating the separate contributions from the outer and middle ears. Unlike other diagnostic tests, SFI can not only evaluate the function of the middle ear, but also assess the function of the outer ear simultaneously in neonates and young infants.

The present study also provided valuable information about the effects of ear canal static pressure on the dynamic behaviour of the outer and middle ear system in neonates (Chapter 3) and this can assist in the interpretation of other middle ear measurements such as tympanometry and identify the negative pressure at which the infant ear canal collapses. The application of both positive and negative static pressure to the ear canal of healthy neonates increased both RF1 and RF2, but decreased the mobility of the outer (ΔSPL1) and middle ear (ΔSPL2). Positive static pressure resulted in lower SPL in the ear canal and negative static pressure resulted in higher SPL in the ear canal than that of ambient pressure (0 daPa), indicating expansion of ear canal volume for positive pressure and reduction of volume for negative pressure. Results also showed that, even at 200 daPa pressures, the ear canal wall of a neonate did not behave like a rigid wall. The study provided additional information on another important factor regarding collapsing ear canals in neonates. It was noted that the ear canal started collapsing at as little as -50 daPa static pressure. At -200 daPa, more than 90% of ears showed signs of collapsed ear canal. Clinically, these results show that tympanometric procedures on newborns should not apply negative pressure to the ear canal beyond -200 daPa because of the possible collapsing ear canal condition. Hence, this collapsing ear canal condition for negative static pressure would render the measurement of peak compensated static admittance using the negative tail compensation method unreliable.

The results of the present study also provided useful clinical information for differentiating healthy ears from ears with middle ear conditions. The resonance that occurred in the higher frequency region (830-1518 Hz) was considered to be associated with movements of the middle ear components. Hence, absence of resonance in the higher frequency (RF2) region with the presence of resonance in the low-frequency (RF1) region in neonates would suggest the possibility of middle ear dysfunction in the affected ear.

7.6.2.2 SFI during diagnostic evaluation of young infants

Developmental factors are important while evaluating the function of the outer and middle ear of young infants of less than 6 months of age. The present study provided normative SFI data for infants at birth, 1, 2, 3, 4, and 6 months of age (Chapter 4). These data could be used as a reference standard for detecting dysfunctions of the conductive pathway in young infants, as age appropriate normative values are required to differentiate age related changes from pathological changes. These normative SFI data can also be used as developmental markers for maturity of the outer ear. The study suggested that ossification of the ear canal might be complete by 6 months of age because of the absence of the outer ear resonance effect.

7.7 Limitations of the study

Although SFI showed promising results as a useful test in assessing neonates and young infants, several limitations have been noted that could affect the clinical application of SFI. First, administering the test battery reference standard with different equipment was time consuming. As the testing time for data collection was limited to certain hours of the day, all testing could not be completed in both ears of all infants recruited for the study. During data collection, only the easily accessible ear was tested first, and the second ear was tested only if time permitted to complete the test and the infant was well settled. This could have led to less number of ears being tested during any given testing session. It is recommended that further studies adopt flexible hours for data collection and recruitment of large numbers of infants.

Second, multiple tests were conducted that involved inserting multiple probes into the ear canal and this disturbed some infants. This shortcoming was partially overcome by testing the infants after feeding when they were well settled. Improvement in instrumentation to

include a single probe assembly to perform multiple tests is recommended. This improvement will also reduce overall testing time and increase the completion rate in testing infants.

Third, the SFI technique requires multiple pressurisations, which may not be desirable in some neonate and infant ears, as pressure changes can be a source of discomfort and require maintaining a probe seal for repeated sweeps. As each sweep is essentially an independent measure, the reliability of relative measures (i.e., comparing SPL curves across different sweeps) may be difficult when probe seal is not maintained and may introduce some artefact. This can be minimised by measuring the loss of pressure before and after the sweep.

Fourth, there was difficulty in measuring RF2 and ΔSPL2, when inflection was small in a few cases. However, this difficulty can be overcome by utilizing an audiologist experienced in SFI measurement. The error can also be reduced by using an automated mathematical procedure after converting all data to a digital format.

Fifth, the present study used a cross-sectional design for measuring developmental characteristics from birth to six months of age, which might have restricted the investigation of maturation of the outer and middle ear. Further studies are recommended using a longitudinal study design to track developmental changes of the outer and middle ear using the SFI technique.

Sixth, a strict protocol for determination of status of the middle ear was used for test battery reference standards. This strict protocol had excluded the ear that passed in one test but referred in the other test within the test battery reference standard. The adoption of this strict protocol did not only reduce the sample size, consequently reducing the power of the statistical analyses, but it also would have inflated the AROC, sensitivity and specificity values for the SFI against the test battery reference standards, especially in evaluating predictive accuracy of SFI technology in identifying conductive conditions in newborns and comparing SFI measures in Australian Aboriginal and Caucasian neonates. In order to overcome these shortcomings, further studies are needed to maximise the recruitment of infants using flexible working hours to collect the data prior to hospital discharge.

Seventh, the sample size was unequal during comparison of SFI between Australian Aboriginal and Caucasian neonates (Chapter 5). The sample size for Australian Aboriginal

neonates was smaller than that of the Caucasian group and, therefore, the findings may not be confidently generalized. Although there was unequal sample size, the power analysis (partial eta squared) showed 0.03 magnitude of effect for RF1 and RF2 which fell between small (0.01) and medium (0.09) effect. However, further studies incorporating a larger sample of Australian Aboriginal neonates and investigating test performance of SFI between Aboriginal and Caucasian neonates are recommended.

Eighth, the results of the study could have been influenced by the pass and refer criteria of some tests. For instance, the pass criterion for HFT was a single positive peak with positive peak admittance (Ya) of ≥0.2 mmho, while double or multiple peaks were considered as a refer in this study. Similarly, the TEOAE criterion of at least a 3 dB SNR in three frequency regions (2000, 3000, and 4000 Hz) and DPOAE criterion of minimum SNR of 6 dB in at least three out of four f2 frequencies (2000, 3000, 4000, and 6000 Hz) might not given optimal results. Furthermore, these pass criteria did not assess frequencies below 2000 Hz where the effect of middle ear dysfunction may be more prominent. Further studies could incorporate 1500 Hz into the protocol to investigate if the inclusion of low frequencies could improve the identification of conductive conditions.

Ninth, test performance of SFI may vary depending on the time of screening during the postnatal period. The mean age at screening of newborns in the present study was 45.6 hrs, but with a substantial standard deviation of 21 hrs. Studies have shown that transient conductive conditions due to the presence of vernix and/or mesenchyme in the outer and middle ear may occur within the first 48 hours of birth (Allen et al., 2005; Doyle et al., 2000; Gravel et al., 2005). The transient conductive conditions would affect referral rates of neonates in all screening tests which in turn affect the test performance of SFI. Further studies should explore the time of testing and compare referral rates and test performance between groups.

Finally, there is lack of a “true” gold standard for testing middle ear function in newborns. Although test battery reference standards were used to determine normal outer/middle ear sound conduction function, a pass in all of these tests cannot definitively rule out slight outer/middle ear dysfunction in newborns (S. Aithal et al., 2012).

7.8 Conclusions

For the first time, the present thesis investigated the use of SFI in neonates and young infants using a battery of tests as reference standards and reported normative SFI data to evaluate outer and middle ear function in neonates and young infants. The study also investigated effects of ear canal static pressure on the dynamic behaviour of the outer and middle ear, compared SFI measures in Australian Aboriginal and Caucasian neonates, reported developmental norms for SFI from birth to six months of age, and measured the predictive accuracy of SFI in identifying conductive condition in neonates. This thesis has enhanced the minimal literature available in relation to the clinical application of the SFI technique in young infants.

From the results of the five studies described in this thesis, the following conclusions can be drawn:

1. Sweep Frequency Impedance (SFI) is a feasible test of outer and middle ear function in neonates. The normative data developed in this study will be useful in evaluating outer and middle ear function in neonates.
2. A test battery approach was used to develop normative SFI data in neonates and young infants in this study. This test battery approach represents the best available reference standard as invasive techniques such as myringotomy or CT scanning under screening setups is not ethical and cannot be used in healthy neonates.
3. The dynamic behaviour of the outer and middle ear under positive pressures is distinctly different to that under negative pressure. Most ears of neonates showed signs of collapse of the ear canal under negative static pressure of -200 daPa.
4. The SFI test provides useful clinical information for differential diagnosis of healthy ears from ears with middle ear conditions.
5. The SFI test is a sensitive test of outer and middle ear function in neonates. It has great promise to be used as a diagnostic test in paediatric clinics.
6. Additionally, the SFI test could be used as a single clinical test with high test performance which is as good as that of a test battery reference standard such as HFT+DPOAE.
7. Australian Aboriginal neonates may have subtle conductive conditions as evidenced by low RF1 and RF2 values measured using SFI. Some Aboriginal

neonates who passed a battery of tests obtained abnormal SFI results, indicating that SFI is more sensitive to outer and middle ear dysfunction than the test battery.

8. The maturation of the outer and middle ear of young infants in the first six months of life could be tracked using SFI. The disappearance of the outer ear resonance by six months of age indicates complete ossification of the ear canal and can be used as a marker for outer ear maturation.

7.9 Directions for future research

Although SFI technology showed great performance in the identification of outer and middle ear function in neonates and young infants, further research is needed before it is used as screening test in UNHS programs or as a diagnostic test in clinics. In the absence of an ideal "gold" standard, the use of a surrogate gold standard such as test battery reference standard or AC and BC ABR may be an ideal option. Further large scale studies incorporating surrogate "gold" standards such as AC and BC ABR to determine the conductive status of the middle ear would improve the accuracy of SFI normative data for neonates and young infants.

The SFI pass and refer criteria were based on the normative range for RF1 and RF2. When response (inflection) was small, it was difficult to measure RF2. In order for SFI to be used as a diagnostic test in a clinical setting, fast, objective and efficient methods for interpreting SFI results need to be developed. There is a need to establish an automated mathematical procedure after converting all data into a digital format to determine pass and refer criteria that could be visually displayed on the screen similar to that used in AABR or automated OAE.

Many studies have reported persistent OME in the neonatal period as a potential predictor of middle ear infections later in life. The present study showed that many Australian Aboriginal neonates have a significant conductive condition at birth. Hence, further longitudinal studies are needed to establish the natural history of conductive conditions due to OME in early infancy in both Australian Aboriginal and Caucasian neonates to determine whether early OME is truly an indicator for later persistent OM. Such studies will be useful in monitoring high risk populations such as Australian Aboriginal children, and also in

development of appropriate public education programs and in management of infants at risk of persistent OM.

The present study showed that Australian Aboriginal neonates had significantly different acoustic-mechanical properties of the outer and middle ear as evidenced by lower outer and middle ear resonance than Caucasian counterparts. However, the reasons for such differences are not known. While it is possible that anatomical and physiological characteristics of the outer and middle ear between two groups could have resulted in differences in SFI findings, the contributing factors cannot be confirmed using the present experimental design. Additional research is required which provide anatomical and physiological data (such as radiological or magnetic resonance imaging) to investigate if differences in the volume of middle ear cavity, are associated with difference in SFI between the two groups.

The present study showed developmental changes in SFI, in the first 6 months of life. The fast maturational process of outer when compared to middle ear was noted in this study. As development of the outer and middle ear continue beyond 6 months of age, further research is needed to establish normative SFI data for different intervals up to 3 years of age to provide additional information on the maturational process. Such normative data will be useful in the differential diagnosis of conductive disorders.

The study showed that SFI can be measured under ambient as well as pressurised conditions. The present study investigated outer and middle ear function under both ambient and pressurised conditions and noted that dynamic behaviour observed under various static ear canal pressure conditions can provide additional clinical information for differential diagnosis of healthy ears from ears with a conductive condition in neonates. Additional studies with large sample sizes are needed to investigate the differences in ears with and without conductive conditions in the developmental time course of the outer and middle ear.

In summary, the present study demonstrated that SFI technology is a feasible test of outer and middle ear function in neonates and young infants, and the normative data established in this study can be used for evaluating outer and middle function in neonates. Future investigations using the SFI technology need to focus on determining the differential diagnosis of middle ear conditions using large samples of infants. Automated and objective

protocols need to be developed to improve the predictive accuracy of the SFI while reducing the test time. Overall, this thesis has provided a substantially original contribution to the knowledge of outer and middle ear assessments in young infants using an innovative technology, the SFI.

7.10 REFERENCES (For Chapter 1 and 7).

AAP. (2004). American Academy of Pediatrics: Diagnosis and management of acute otitis media. *Pediatrics, 113*, 1451-1465.

ABS. (2010). *Australian Bureau of Statistics: 4704.0 The Health and Welfare of Australia's Aboriginal and Torres Strait Islander Peoples.*

Aithal, S., Aithal, V., & Katrina, R. (2008). *Conductive hearing loss in neonates- Referral from newborn hearing screens as presentation for middle ear pathlogy in newborn population*. Paper presented at the Northern Exposure Symposium, 25th to 27th August, Townsville.

Aithal, S., Aithal, V., & Katrina, R. (2009). *Conductive hearing loss in neonates*. Paper presented at the Second Australasian newborn hearing screening conference, 21st to 23rd May, Adelaide.

Aithal, S., Aithal, V., & Kei, J. (2017). Effect of ear canal pressure and age on wideband absorbance in young infants. *International Journal of Audiology*. doi:https://doi.org/10.1080/14992027.2017.1284352

Aithal, S., Aithal, V., Kei, J., & Driscoll, C. (2012). Conductive hearing loss and middle ear pathology in young infants referred through universal newborn hearing screening program in Australia. *Journal of American Academy of Audioogy, 23*, 673-685.

Aithal, S., Kei, J., & Driscoll, C. (2014). Wideband reflectance in young infants (0-6 months): A cross-sectional study. *Journal of American Academy of Audiology, 25*, 471-478.

Aithal, S., Kei, J., Driscoll, C., & Khan, A. (2013). Normative wideband reflectance measures in healthy neonates. *International Journal of Pediatric Otorhinolaryngoogy, 77*, 29-35. Retrieved from http://dx.doi.org/10.1016/j.ijporl.2012.09.024

Aithal, S., Kei, J., Driscoll, C., Khan, A., & Swanston, A. (2015). Wideband Absorbance Outcomes in Newborns: a comparison with high-frequency tympanometry, automated

brainstem response, and transient evoked and distortion product otoacoustic emissions. *Ear and Hearing, 36*, e237-e250.

Aithal, V., Kei, J., Driscoll, C., Swanston, A., Roberts, K., Murakoshi, M., & Wada, H. (2014). Normative sweep frequency impedance measures in healthy neonates. *Journal of American Academy of Audioogy, 25*, 343-354.

Allen, J. B., Jeng, P. S., & Levitt, H. (2005). Evaluation of human middle ear function via an acoustic power assessment. *Journal of Rehabilitation Research and Development, 42 (suppl.2)*, 63-77.

ANHSC. (2001). *Australian National Hearing Screening Committee. Australian consensus statement on universal neonatal hearing screening*. Adelaide.

Anson, B. J., & Donaldson, J. A. (1981). *Surgical Anatomy of the Temporal Bone and Ear*. Philadelphia: Saunders.

Antonio, S. M., Don, D., Doyle, W. J., & Alper, C. M. (2002). Daily home tympanometry to study the pathogenesis of otitis media. *Paediatric Infectious Disease Journal, 21*, 882-885.

ASHA. (2004). *Guidelines for the audiologic assessment of children from birth to 5 years of age*.

Bachman, K. R., & Arvedson, J. C. (1998). Early identification and intervention for children who are hearing impaired. *Pediatric Review, 19*, 155-165.

Baldwin, M. (2006). Choice of probe tone and classification of trace patterns in tympanometry undertaken in early infancy. *International Journal of Audiology, 45*, 417-427.

Balkany, T. J., Berman, S. A., Simmons, M. A., & Jafek, B. W. (1978). Middle ear effusions in neonates. *The Laryngoscope, 88*, 398-405.

Boone, R. T., Bower, C. M., & Martin, P. F. (2005). Failed newborn hearing screens as presentation of otitis media with effusion in the newborn population. *International Journal of Pediatric Otorhinolaryngology, 69*, 393-397.

Boswell, J. (1994). *A prospective audiological study of otitis media in Aboriginal and non-Aboriginal Australian Infants.* (PhD thesis), University of Sydney, Sydney.

Boswell, J., & Nienhuys, T. (1995). Onset of otitis media in the first eight weeks of life in aboriginal and non-aboriginal australian infants. *Annals of Otology Rhinology and Laryngology, 104*, 542-549.

Boswell, J., & Nienhuys, T. (1996). Patterns of persistent otitis media in the first year of life in aboriginal and non-aboriginal infants. *Annals of Otology Rhinology and Laryngology, 105*, 893-900.

Boswell, J., Nienhuys, T., Rickards, F. W., & Mathews, J. D. (1993). Onset of otitis media in Australian Aboriginal infants in a prospective study from birth. *Australian Journal of Otolaryngology, 1*, 232-237.

Boudewyns, A., Declau, F., Van den Ende, J., Van Kerschaver, E., Dirckz, S., Hofkens-Van den Brandt, A., & Van de Heyning, P. (2011). Otitis media with effusion: an underestimated cause of hearing loss in infants. *Otology and Neurotology, 32*, 799-807.

Brooks, D. N. (1968). An objective method of determining fluid in the middle ear. *International Journal of Audiology, 7*, 280-286.

Buch, N. H., & Jorgensen, M. B. (1964). Embroynic connective tissue in the tympanic cavity of the fetus and newborn. *Acta Otolaryngologica, 58*, 111-126.

CAHS Western Australia. (2011). Newborn Hearing Screening Program (WA). Retrieved from http://www.pmh.health.wa.gov.au/services/newborn_hearing/index.htm

Casselbrant, M. L., Brostoff, L. M., Cantekin, E. L., Flaherty, M. R., Doyle, W. J., & Bluestone, C. D. (1985). Otitis media with effusion in preschool children. *Laryngoscope, 95*, 428-436.

Casselbrant, M. L., Mandel, E. M., Kurs-Lasky, M., Rockette, H. E., & Bluestone, C. D. (1995). Otitis media in a population of black American and white American infants, 0-2 years of age. *International Journal of Pediatric Otorhinolaryngology, 33*, 1-16.

Casselbrant, M. L., Mandel, E. M., Rockette, H. E., & Bluestone, C. D. (2003). *Incidence of otitis media and bacteriology of acute otitis media during the first two yeaes of life.* Paper presented at the Recent advances in otitis media with effusion, Philadelphia.

Cavanaugh, R. M. (1987). Pneumatic otoscopy in healthy full-term infants. *Pediatrics, 79*, 520-523.

Cavanaugh, R. M. (1989). Pediatricians and the pneumatic otoscope: Are we playing it by ear? *Pediatrics, 84*, 362-364.

CDCP. (2003). Centers for Disease Control Prevention. Infants tested for hearing loss - Unites States, 1999-2001. MMWR. . *Morbidity & Mortality Weekly Report, 52*, 981-984.

Chang, K. W., Vohr, B. R., Norton, S. J., & Lekas, M. D. (1993). External and middle ear status related to evoked otoacoustic emission in newborns. *Archives of Otolaryngology - Head &Neck Surgery, 119*, 276-282.

Chang, P., Moffat, D. A., & Baguley, D. (2000). Universal neonatal hearing screening - current review. *Australian Journal of Otolaryngol, 3*, 543-550.

Choo, D., & Meinzen-Derr, J. (2010). Universal newborn hearing screening 2010. *Current Opinion in Otolaryngology & Head and Neck Surgery., 18*, 399-404.

Clark, J. L., Roeser, R. J., & Mendrygal, M. (2007). Middle ear measures. In R. J. Roeser, M. Valente, & H. Hosford-Dunn (Eds.), *Audiology Diagnosis* (2nd ed., pp. 380-399). New York: Thieme.

Clemens, C. J., & Davis, A. (2001). Minimizing false positive in universal newborn hearing screening: A simple solution. *Pediatrics, 107*, 1-3.

Clemens, C. J., Davis, A., & Bailey, A. R. (2000). The False-positive in universal newborn hearing screening. *Pediatrics, 106*, 1-5.

Colletti, V. (1976). Tympanometry from 200 to 2000 Hz probe. *Audiology, 15,*106-119.

Colletti, V. (1977). Multifrequency tympanometry. *Audiology, 16*, 278-287.

Currie, K. (2005). *Review of evidence of good/best practice for early childhood intervention services for children with a diagnosed hearing impairment*. Melbourne: Monash University Retrieved from http:/www.med.monash.edu.au/healthservices/cce.

de Jonge, R. R. (1986). Normal tympanometric gradient: A comparison of three methods. *Audiology, 25*, 299-308.

Derkay, C. S., Bluestone, C. D., Thompson, A. E., Stephenson, J. S., & Kardtzke, D. (1988). Otitis media in the pediatric intensive care unit; A prospective study of its incidence, prevalence, bacteriology, etiology and treatment *Annals of Otology Rhinology and Laryngology, 97 (suppl) 133*, 56-57.

deSa, D. J. (1973). Infection and amniotic aspiration of middle ear in stillbirths and neonatal deaths. *Archives of Disease in Childhood, 48*, 872-880.

deSa, D. J. (1983). Mucosal metaplasia and chronic inflammation in the middle ear of infants receiving intensive care in the neonatal period. *Archives of Disease in Childhood, 58*, 24-28.

Dhar, S., & Hall, J. W. (2012). *Otoacoustic Emissions: Principles, procedures, and protocols*. San Diego: Plural.

Diefendorf, A. O. (1999). Screening fro hearing loss in infants. *Volta Review, 99*, 43-61.

Douglas, F., & Powers, J. (1989). *Ear disease in infancy in three rural Aboriginal communities.68-69,* Menzies School of Health Research Annual Report, Darwin.

Doyle, K. J., Burggraaff, B., Fujikawa, S., Kim, J., & Macarthur, C. J. (1997). Neonatal hearing screening with otoscopy, auditory brainstem response and otoacoustic emissions. *Otolaryngology - Head & Neck Surgery, 116*, 597-603.

Doyle, K. J., Kong, Y. Y., Strobel, K., Dallaire, P., & Ray, R. M. (2004). Neonatal middle ear effusion predicts chronic otitis media with effusion. *Otology and Neurotology, 25*, 318 - 322.

Doyle, K. J., Rodgers, P., Fujikawa, S., & Newman, E. (2000). External and middle ear effects on infant hearing screening test results. *Otolaryngolology- Head and Neck Surgery, 122*, 477-481.

Driscoll, C., Kei, J., & McPherson, B. (2001). Outcomes of transient evoked otoacoustic emission testing in six year old school children: A comparision with pure tone screening and tympanometry. *International Journal of Pediatr Otorhinolaryngology, 57*, 67-76.

Eavey, R. (1993). Abnormalities of the neonatal ear: otoscopic observations, histologic observations and a model for contamination of the middle ear by cellular contents of amniotic fluid. *Laryngoscope, 103*, 1-33.

Eavey, R., Camacho, A. R., & Northrop, C. C. (1992). Chronic ear pathology in a model of neonatal amniotic fluid ear inoculation. *Archives of Otolaryngology Head Neck Surgery, 118*, 1198-1203.

Eby, T. L., & Nadol, J. B. (1986). Postnatal growth of the human temporal bone- implications for cochlear implants in children. *Annals of Otology Rhinology and Laryngology, 95*, 356-364.

Eliachar, I., & Northern, J. L. (1974). Studies in tympanometry: Validation of the present technique for determining intra-tympanic pressures through the intact ear drum. *Laryngoscope, 84*, 247-255.

Feeney, M. P., & Sanford, C. A. (2012). Application of wideband acoustic transfer functions to the assessment of the infant ear. In J. Kei & F. Zhao (Eds.), *Assessing middle ear function in infants* (pp. 131-161). San Diego: Plural.

Feldman, A. S. (1974). Eardrum abnormalities and measurement of middle ear function. *Archives of Otolaryngology, 99*, 211-217.

Fiellau-Nikolajsen, M. (1983). Tympanometry and secretory otiti media . Obervations on diagnosis, epidemiology, treatment, and prvention in prospective cohort studies of three year old children. *Acta Otolaryngologica, 394 (Suppl.)*, 1-73.

Finitzo, T., Albright, K., & O'Neal, J. (1998). The newborn with hearing loss: Detection in the nursery. *Pediatrics, 102*, 1452-1460.

Finitzo, T., Friel-Patti, S., Chinn, K., & Orval, B. (1992). Tympanometry and otoscopy prior to myringotomy: Issues in diagnosis of otitis media. *International Journal of Pediatric Otorhinolaryngology, 24*, 101-110.

Foreman, A. E. W., Boswell, J. B., & Mathews, J. (1992). *Prevalence of otitis media in Aboriginal children of pre-school age in three Northern Territory rural communities.* Menzies School of Health Research Annual Report, Darwin.

Fortnum, H., & Davis, A. (1997). Epidemiology of permanent hearing impairment in Trent region, 1985-1993. *British Journal of Audiology, 31*, 409-446.

Fowler, C. G., & Shanks, J. E. (2002). Tympanometry. In J. Katz (Ed.), *Handbook of clinical audiology* (pp. 175 - 204). Baltimore: Lippincott Williams & Wilkins.

Fria, T. J., Cantekin, E. I., & Eichler, J. A. (1985). Hearing acuity of children with otitis media with effusion. *Archives of Otolaryngology, 111*, 10-16.

Funasaka, S., Funai, H., & Kumakawa, K. (1984). Sweep-frequency tympanometry: Its development and diagnostic value. *Audiology, 23*, 366-379.

Funasaka, S., & Kumakawa, K. (1988). Tympanometry using a sweep-frequency probe tone and its clinical evaluation. *Audiology, 27*, 99-108.

Glennon, S. (2007). *Healthy hearing program: A state wide neonatal hearing screening program*. Queensland Health, Brisbane.

Gorga, M. P., Neely, S. T., Bergman, B. M., Beauchaine, K. L., Kaminski, J. R., Perters, J., . . . Jesteadt, W. (1993). A comparision of transient evoked and distortion product otoacoustic emissions in normal hearing and hearing impaired subjects. *Journal of Acoustical Society of America, 94*, 2639-2648.

Gravel, J. S., White, K. R., Johnson, J. L., Widen, J. E., Vohr, B. R., James, M., . . . Meyer, S. (2005). A multisite study to examine the efficacy of the otoacoustic emission/automated auditory brainstem response newborn hearing screening protocol: recommendations for policy, practice and research. *American Journal of Audiology, 14*, S217-S228.

Guggenheim, P., Clements, L., & Schlesinger, A. (1956). The significance and fate of the mesenchyme of the middle ear. *Laryngoscope, 66*, 1303-1326.

Hall, J. W., Smith, S. D., & Popelka, G. R. (2004). Newborn hearing screening with combined otoacoustic emissions and auditory brainstem responses. *Journal of American Academy of Audiology, 15*, 414-425.

Himelfarb, M. Z., Popelka, G. R., & Shanon, E. (1979). Tympanometry in normal neonates. *Journal of Speech & Hearing Research, 22*, 179-191.

Hocke, T., Eiber, A., Vorwerk, U., Pethe, J., Muhler, R., Specht, H. v., & Begall, K. (2000). Resonant frequency pattern in multifrequency tympanograms: Results in normally - hearing subjects. *Audiology, 39*, 119-124.

Hoffman, H. J., Park, J., & Losonczy, K. G. e. a. (2007). *Risk factors, treatments, and other conditions associated with frequent ear infections in US children through 2 years of age: the early childhood longitudinal study-bithr cohort (ECLS-B)*. Paper presented at the 9th International symposium on recent advances in otitis media, Saint pete Beach.

Holster, I. L., Hoeve, L. J., Wieringa, M. H., Willis-Lorier, R. M. S., & de Gier, H. H. W. (2009). Evaluation of hearing loss after failed neonatal hearing screening. *Journal of Pediatrics, 155*, 646-650.

Holte, L., Cavanaugh, R. M., & Margolis, R. H. (1990). Ear canal wall mobility and tympanometric shape in young infants. *Journal of Pediatrics, 117*, 77-80.

Holte, L., Margolis, R. H., & Cavanaugh, R. M. (1991). Developmental changes in multifrequency tympanograms. *Audiology, 30*, 1-24.

Hunter, L. L., & Blankenship, C. M. (2017). Middle ear measurement. In A. Tharpe, M. & R. Seewald (Eds.), *Comprehensive handbook of pediatric audiology* (2nd ed.). San Diego: Plural Publishing.

Hunter, L. L., Feeney, M. P., Miller, J. A. L., Jeng, P. S., & Bohning, S. (2010). Wideband reflectance in newborns: normative regions and relationship to hearing-screening results. *Ear and Hearing, 31*, 599-610.

Hunter, L. L., & Margolis, R. H. (1992). Multifrequency tympanometry: current clinical application. *American Journal of Audiology, 1*, 33-43.

Hunter, L. L., & Margolis, R. H. (2011). Middle ear measurement. In R. Seewald & A. Tharpe, M. (Eds.), *Comprehensive handbook of pediatric audiology* (pp. 365-388). San Diego: Plural.

Hunter, L. L., Margolis, R. H., Daly, K. A., & Giebink, G. S. (1992). *Relationship of tympanometric estimates of middle ear volume to middle ear status at surgery.* Paper presented at the Abstracts of the midwinter research meeting of the association for research in otolaryngology, St. Petersburg Beach, FL.

Hunter, L. L., Prieve, B. A., Kei, J., & Sanford, C. A. (2013). Pediatric applications of wideband acoustic immittance measures. *Ear and Hearing, 34*(Supplement 1), 36S-42S.

Ikui, A., Sando, I., Haginomori, S., & Sudo, M. (2000). Postnatal development of the tympanic cavity: A computer-aided reconstruction and measurement study. *Acta Otolaryngologica, 120*, 375-379.

Jaffe, F. B., Hurtado, F., & Hurtado, E. (1970). Tympanic membrane mobility in the newborn (with seven months' follow-up). *Laryngoscope, 80*, 36-48.

Jaisinghani, V. J., Paparella, M. M., Schachern, P. A., & Le, C. T. (1999). Residual mesenchyme persisting into adulthood. *American Journal of Otolaryngology, 20*, 363-370.

JCIH. (2007). Joint Committee on infant Hearing. Year 2007 Position statement: Principlies and guidelines for early hearing detection and intervention programs. *Pediatrics, 120*, 898-921.

Jerger, J. (1970). Clinical experience with impedance audiometry. *Archives of Otolaryngology, 92*, 311-324.

Jerger, J., Jerger, S. J., & Mauldin, L. (1972). Studies in impedance audiometry: I. Normal and sensorineural ears. *Archives of Otolaryngology, 96*, 513-523.

Keefe, D. H., Bulen, J. C., Arehart, K. H., & Burns, E. M. (1993). Ear canal impedance and reflection coefficient in human infants and adults. *Journal of Acoustical Society of America, 94*(5), 2617-2638.

Keefe, D. H., & Feeney, M. P. (2009). Principles of acoustic imittance and acoustic transfer functions. In J. Katz, R. F. Burkhard, & L. Medwetsky (Eds.), *Handbook of clinical audiology*. Baltimore: Lippincott, Williams and Wilkins.

Keefe, D. H., Folsom, R. C., Gorga, M. P., Vohr, B. R., Bulen, J. C., & Norton, S. J. (2000). Identification of neonatal hearing impairment: ear-canal measurements of acoustic admittance and reflectance in neonates. *Ear and Hearing, 21*, 443-461.

Keefe, D. H., Sanford, C. A., Ellison, J. C., Fitzpatrick, D. F., & Gorga, M. A. (2012). Wideband aural acoustic absorbance predicts conductive hearing loss in children. *International Journal of Audiology, 51*, 880-891.

Keefe, D. H., Zhao, F., Neely, S. T., Gorga, M. P., & Vohr, B. R. (2003). Ear-canal acoustic admittance and reflectance effects in human neonates. I. Predictions of otoacoustic emission and auditory brainstem responses. *Journal of Acoustical Society of America, 113*(1), 389-406.

Kei, J., Allison-Levick, J., Dockray, J., Harrys, R., Kirkegard, C., Wong, J., . . . Tudehope, D. (2003). High-frequency (1000 Hz) tympanometry in normal neonates. *Journal of American Academy of Audiology, 14*, 20-28.

Kei, J., Hourigan, A., Moore, K., Keogh, T., & Driscoll, C. (2005). Tympanometric findings in children at school entry: A normative study. *Australian and New Zealand Journal of Audiology,* 27, 89-96.

Kei, J., & Mazlan, R. (2012). High-frequency tympanometry: Clinical applications. In J. Kei & F. Zho (Eds.), *Assessing middle ear function in infants* (pp. 39-67). San Diego: Plural.

Kei, J., Mazlan, R., Seshimo, N., & Wada, H. (2010). *Measuring middle ear resonant frequency in neonates: A preliminary study*. Paper presented at the XIX National Conference of Audiological Society of Australia, Sydney.

Kei, J., Sanford, C. A., Prieve, B. A., & Hunter, L. L. (2013). Wideband acoustic immittance measures: developmental characteristics (0 to 12 months). *Ear and Hearing, 34*(Supplement 1), 17S-26S.

Kei, J., & Zhao, F. (2012). The challenge of assessing middle ear function in young infants: Summary and future directions. In J. Kei. & F. Zhao. (Eds.), *Assessing middle ear function in infants* (pp. 163-177). San Diego: Plural.

Kemp, D. T. (1979). Evidence of mechanical nonlinearity and freqeuncy selective wave amplification in the cochlea. *Archives of Otorhinolaryngology, 244*, 37-45.

Kemp, D. T., Ryan, S., & Bray, S. (1990). A guide to effective use of otoacoustic emissions. *Ear and Hearing, 11*, 93-105.

Kemper, A. R., & Downs, S. M. (2000). A cost-effective analysis of newborn hearing screening strategies. *Archives of Pediatric Adolescent Medicine, 154*, 484-488.

Kenna, M. A. (1990). Embryology and developmental anatomy of the ear. In C. D. Bluestone, S. E. Stool, & M. D. Sheetz (Eds.), *Pediatric Otolaryngology* (2nd Edition ed., pp. 77-87). Philadelphia: Saunders.

Koebsell, K. A., & Margolis, R. H. (1986). Tympanometric gradient measured from normal preschool children. *Audiology, 25*, 149-157.

Kok, M. R., vanZanten, G. A., & Brocaar, M. P. (1992). Growth of evoked otoacoustic emissions during the first days postpartum. *Audiology, 31*, 140-149.

Kokko, E. (1974). Chronic secretory otitis media in children. *Acta Otolaryngology, 327(Suppl)*, 1-44.

Kuypers, L. C., Decraemer, W. C., & Dirckx, J. J. (2006). Thickness distribution of fresh and perserved human eardrums measured with confocal microscopy. *Otology and Neurotology, 27*(2), 256-264.

laRossa, M. M., Mitchell, S., & Cardinal, J. W. (1993). Tympanometry as a screenign tool in the NICU: Is it effective? *Neonatal Network, 12*, 33-35.

Leach, A. J. (1996). *Prospective studies of respiratory pathogens, particularly Streptococcus pneumoniae in Aboriginal and non-Aboriginal infants: impact of antibiotic use and implications for otitis media.* (PhD thesis), University of Sydney, Sydney.

Leach, A. J. (1999). Otitis media in Australian Aboriginal children: an overview. *International Journal of Pediatric Otorhinolaryngology, 49*, S173-S178.

Leach, A. J., Boswell, J., Asche, V., Nienhuys, T., & Mathews, J. (1994). Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian Aboriginal infants. *Pediatric Infectious Diseases Journal, 13*, 983-989.

Lee, D. H. (2010). How to improve the accuracy of diagnosing otitis media with effusion in a paediatric population. *International Journal of Pediatric Otorhinolaryngology, 74*, 151-153.

Lee, D. H., & Yeo, S. W. (2004). Clinical diagnostic accuracy of otitis media with effusion in children and significance of myringotomy: diagnostic or therapeutic? *Journal of Korean Medical Science, 19*, 739-743.

Lehmann, D., Weeks, S., Jacoby, P., Elsbury, D., Finucane, J., Stokes, A., . . . Coates, H. (2008). Absent otoacoustic emissions predict otitis media in young Aboriginal children: a birth cohort study in Aboriginal and non-Aboriginal children in an arid zone of Western Australia *BMC Pediatrics, 8*, 32- 43.

Liden, G. (1969). Tests for stapes fixation. *Archives of Otolaryngology, 89*, 215-219.

Liden, G., Harford, E., & Hallen, O. (1974). Automatic tympanometry in clinical practice. *Audiology, 13*, 126-139.

Lindeman, P., & Holmquist, J. (1982). Volume measurement of middle ear and mastoid air cell system with impedance audiometry on patients with ear drum perforation *Acta Otolaryngologica, 386 (Suppl.)*, 70-73.

Marchant, C. D., McMillan, P. M., Shurin, P. A., Johnson, C. E., Turczyk, V. A., Feinstein, J. C., & Panek, D. M. (1986). Objective diagnosis of otitis media in early infancy by tympanometry and ipsilateral acoustic reflex thresholds. *Journal of Pediatrics, 109*, 590-595

Margolis, R. H. (2002). Influence of middle ear disease on otoacoustic emissions. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic Emissions: Clinical Applications* (pp. 190-212). NY: Thieme.

Margolis, R. H., Bass-Ringdahl, S., Hanks, W. D., Holte, L., & Zapala, D. A. (2003). Tympanometry in newborn infants- 1 kHz norms. *Journal of American Academy of Audiology, 14*, 383-392.

Margolis, R. H., & Goycoolea, H. G. (1993). Multifrequency tympanometry in normal adults. *Ear and Hearing, 14*, 408-413.

Margolis, R. H., & Heller, J. W. (1987). Screening tympanometry: criteria for medical referral. *Audiology, 26*, 197-208.

Margolis, R. H., & Hunter, L. L. (2000). Acoustic immittance measurements. In R. J. Roeser, V. Michael, & H. Hosford-Dunn (Eds.), *Audiology Diagnosis* (pp. 381-423). New York: Thieme.

Margolis, R. H., & Nelson, D. A. (1992). Acute otitis media with transient sensorineural hearing loss: A case study. *Archives of Otolaryngology-Head and Neck Surgery, 119*, 682-686.

Margolis, R.H., Van Camp. K.J., Wilson, R.H., & Creten, W.L. (1985). Multifrequency tympanometry in normal ears. *Audiology,* 24, 44-53.

Mason, J. A., & Herrman, K. R. (1998). Universal infant hearing screening by automated auditory brainstem response measurement. *Pediatrics, 101*, 221-228.

Mazlan, R., & Kei, J. (2012). Measuring middle ear function in young infants: An overview. In J. Kei & F. Zhao (Eds.), *Assessing middle ear functions in infants* (pp. 17-37). San Diego: Plural.

Mazlan, R., Kei, J., & Hickson, L. (2009). Test-retest reliability of the acoustic stapedial test in healthy neonates. *Ear and Hearing, 30*, 295-301.

McCafferty, G. J., Lewis, A. N., Coman, W. B., & Mills, C. A. (1985). A nine year study of ear disease in Australian Aboriginal children. *Journal of Laryngology and Otology, 99*, 117-125.

McHugh, G. R., & Traynor, R. M. (2009). Video otomicroscopy: A new "vision" in cerumen management. *Hearing Review*. Retrieved from http://www.hearingreview.com/issues/articles/2009-05_01.asp

McKinley, A. M., Grose, J. H., & Roush, J. (1997). Multifrequency tympanometry and evoked Otoacoustic emissions in neonates during the first 24 hours of life. *Journal of American Academy of Audiology, 8*, 218-223.

McLellan, M. S., & Webb, C. H. (1957). Ear studies in the newborn infant. *Journal of Pediatrics, 51*, 672-677.

Mehl, A. L., & Thomson, V. (1998). Newborn hearing screening; The great Omission. *Pediatrics, 101;e4*, 1-10. Retrieved from http:pediatrics.aappublications.org/content/101/1/e4.full.html

Merchant, G. R., Horton, N. J., & Voss, S. E. (2010). Normative reflectance and transmittance measurements on healthy newborn and 1-month-old infnats. *Ear and Hearing, 31*, 1-9.

Meyer, S. E., Jardine, C. A., & Deverson, W. (1997). Developmental changes in tympanometry: A case study. *British Journal of Audiology, 31*, 189-195.

Moran, D. J., Waterford, J. E., Hollows, F., & Jones, D. L. (1979). Ear disease in rural Australia. *Medical Journal of Australia, 2*, 210-212.

Morris, P. S., Leach, A. J., & Silberberg, P. e. a. (2005). Otitis media in young Aboriginal children from remote communities in Northern and Central Australia: a cross-sectional survey. *BMC Pediatrics, 5*, 27.

MRC. (2003). MRC multicentre otitis media study group. The role of ventilation tube status in the hearing levels in children managed for bilateral persistent otitis media with efusion. *Clinics in Otolaryngology and Allied Sciences, 28*, 146-153.

Murakoshi, M., Yoshida, N., Sugaya, M., Ogawa, Y., Hamanishi, S., Kiyokawa, H., . . . Wada, H. (2013). Dynamic characteristics of the middle ear in neonates. *International Journal of Pediatric Otorhinolaryngology, 77*, 504-512.

Murakoshi, M., Zhao, F., & Wada, H. (2012). Current developments in the clinical application of the sweep frequency impedance (SFI) in assessing middle ear dysfunction. In J. Kei. & F. Zhao. (Eds.), *Assessing middle ear function in infants* (pp. 107-129). San Diego: Plural.

Northrop, C., Piza, J. E., & Eavey, R. (1986). Histological observations of amniotic fluid cellular content in the ears of neonates and infants. *International Journal of Pediatric Otorhinolaryngology, 11*, 113-127.

Northrop, C., Piza, J. E., Karmody, C. S., & Eavey, R. (1999). *The neonatal middle ear. Indicator for future problems?* Paper presented at the Third extraordinary symposium on recent advances in Otitis Media, Cophenhagen.

Norton, S. J., Gorga, M. P., Widen, J. E., Folsom, R. C., Sininger, Y., Cone-Wesson, B., . . . Fletcher, K. A. (2000b). Identification of neonatal hearing impairment: Evaluation of transient evoked otoacoustic emission, distortion prodcut otoacoustic emission and auditory brainstem response test performance. *Ear and Hearing, 21*, 508-528.

Nozza, R. J., Bluestone, C. D., Kardtzke, D., & Bachman, K. R. (1992). Towards the validation of aural acoustic immittance measures for diagnosis of middle ear effusion in children. *Ear and Hearing, 13*, 442-453.

Nozza, R. J., Bluestone, C. D., Kardtzke, D., & Bachman, K. R. (1994). Identification of middle ear effusion by aural acoustic admittance and otoscopy. *Ear and Hearing, 15*, 310-323.

Ostergard, C. A., & Carter, D. R. (1981). Positive middle ear presure shown by tympanometry. *Archives of Otolaryngology, 107*, 353-356.

Palva, T., Northrop, C., & Ramsay, H. (1999). Spread of amniotic fluid cellular content within the neonate middle ear. *International Journal of Pediatric Otorhinolaryngology, 48*, 143-153.

Palva, T., Northrop, C., & Ramsay, H. (2001). Foreign body neonatal otitis media in infants. *Otology and Neurotology, 22*, 433-443.

Paradise, J. L. (1981). Otits media during early life: how hzardous to development? A critical review of the evidence. *Pediatrics, 68*, 869-873.

Paradise, J. L., Rockette, H. E., Colborn, D. K., Bernard, B. S., Smith, C. G., Kurs-Lasky, M., & Janosky, J. E. (1997). Otitis midia in 2253 Pittsburgh area infants: Prevalence and risk factors during the first two years of life. *Pediatrics, 99*, 318-333.

Paradise, J. L., Smith, C. G., & Bluestone, C. D. (1976). Tympanometric detecion of middle ear effusion in infants and young children. *Pediatrics, 58*, 198-210.

Piza, J. E., Gonzalez, M. P., Northrop, C. C., & Eavey, R. D. (1989). Meconium contaminatin of the neonatal middle ear. *Journal of Pediatrics, 115*(6), 910-914.

Prieve, B. A., Dalzell, L., Berg, A., Bradley, M., Cacace, A., & Campbell, D. e. a. (2000). The New York state universal newborn hearing screening demonstration project: Outpatient outcome measures. *Ear and Hearing, 21*, 104-117.

Prieve, B. A., Feeney, M. P., Stenfelt, S., & Shahnaz, N. (2013). Prediction of conductive hearing loss using wideband acoustic immittance. *Ear and Hearing, 34*, 54s-59s.

Prieve, B. A., Gorga, M. P., Ascmidt, A., Neely, S., Peters, I., Schulters, L., & Jesteadt, W. (1993). Analysis of transient evoked otoacoustic emissions in normal hearing and hearing impaired ears. *Journal of Acoustical Society of America, 93*, 3308-3319.

Priner, R., Freeman, S., Perez, R., & Sohmer, H. (2003). The neonate has a temporary conductive hearing loss due to fluid in the middle ear. *Audiology NeuroOtology, 8*, 100-110.

Proctor, B. (1967). Embryology and anatomy of the eustachian tube. *Archives Otolaryngology, 86*, 503-514.

Proctor, L. R., & Kennady, D. W. (1990). High-risk newborns who failed hearing screening: Implications of otological problems. *Seminar in Hearing, 11*, 167-176.

Purdy, S. C., & Williams, M. J. (2002). High freqeuncy tympanometry: A valid and reliable immittance test protocol for young infnats. *New Zealand Audiological Society Bulletin, 10*, 9-24.

Qi, L., Funnell, W. R., & Daniel, S. J. (2008). A nonlinear finite-element model of the newborn middle ear. *Journal of Acoustical Society of America, 124*, 337-347.

Qi, L., Liu, H., Lutfy, J., Funnell, W. R., & Daniel, S. J. (2006). A nonlinear finite-element model of the newborn ear canal. *Journal of Acoustical Society of America, 120*, 3789-3798.

Ramsay, H., Palva, T., & Northrop, C. C. (2001). Spread and fate of amniotic fluid cellular content in the middle ear. *Acta Otolaryngologica, 121*(2), 190-193.

Rebgetz, P. T. E., Trennery, P., Powers, J., & Mathews, J. (1989). *Incidence of otitis media in the first year of life.* Menzies School of Health Research Annual Report, Darwin.

Renvall, U., & Holmquist, J. (1976). Tympanometry revealing middle ear pathology. *Annals of Otology Rhinology and Laryngology, 85*, 209-215.

Rhodes, M. C., Margolis, R. H., Hirsch, J. E., & Napp, A. P. (1999). Hearing screening in the newborn intensive care nursery: comparison of methods. *Otolaryngology- Head and Neck Surgery, 120*, 799-808.

Roberts, D. G., Johnson, C. E., Carin, S. A., Turczyk, V., Karnuta, M. A., & Yaffee, K. (1992). Resolution of middle ear effusion in newborns. *Archives of Pediatric Adolesccnt Medicine, 149*, 873-877.

Rosenfeld, R. M., Culpepper, L., Doyle, K. J., Grundfast, K., Hoberman, A., Kenna, M., . . . Woods Jr, C. (2004). Clinical practice guideline: otitis media with effusion. *Otolaryngology- Head and Neck Surgery, 130*, 95-118.

Roush, J., Bryant, K., M., M., Zeisel, S., & Roberts, J. (1995). Developmental changes in static admittance and tymapnometric width in infants and toddlers. *Journal of American Academy of Audiology, 6*, 334-338.

Ruah, C. B., Schachern, P. A., Zelterman, D., Paperella, M., & Yoon, T. (1991). Age related morphologic changes in the human tympanic membrane. A light and electron microscopic study. *Archives of Otolaryngology- Head and Neck Surgery, 117*, 627-634.

Sabo, D. L., Paradise, J. L., Kurs-Lasky, M., & Smith, G. C. (2003). Hearing levels in infants and young children in relation to testing technique, age group, and the presence or absence of middle ear effusion. *Ear and Hearing, 24*, 38-47.

Sanford, C. A., & Brockett, J. E. (2014). Characteristics of wideband acoustic immittance in patients with middle ear dysfunction. *Journal of American Academy of Audiology, 25*, 425-440.

Sanford, C. A., & Feeney, M. P. (2008). Effects of maturation on tympanometric wideband acoustic transfer functions in human infants. *Journal of Acoustical Society of America, 124*(4), 2106-2122.

Sanford, C. A., Keefe, D. H., Liu, Y.-W., Fitzpatrick, D. F., McCreery, R. W., Lewis, D. E., & Gorga, M. P. (2009). Sound-conduction effects on Distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry. *Ear and Hearing, 30*, 635-652.

Sangster, L. (2011). Critical review: Can wideband energy reflectance be used in newborn hearing screening to detect transient middle ear dysfunction and to interpret screening results? University of Western Ontario, Canada.

Saunders, J. C., Kaltenback, J. A., & Relkin, E. M. (1983). The structural and functional development of the outer and middle ear. In R. Romand & M. R. Romand (Eds.), *Development of Auditory and Vestibular systems* (pp. 3-25). New York: Academic Press.

Shahnaz, N. (2008). Wideband reflectance in neonatal intensive care units. *Journal of American Academy of Audiology, 19*(5), 419-429.

Shahnaz, N., Miranda, T., & Polka, L. (2008). Multifrequency tympanometry in neonatal intensive care unit and well babies. *Journal of American Academy of Audiology, 19*, 392-418.

Shahnaz, N., & Polka, L. (1997). Standard and multifrequency tympanometry in normal and otosclerotic ears. *Ear and Hearing, 18*, 326-341.

Shanks, J. E., & Shohet, J. (2009). Tympanometry in clinical practice. In J. Katz, L. Medwetsky, R. Burkard, & L. Hood (Eds.), *Handbook of clinical audiology* (pp. 157-188). Baltimore: Lippincott Williams & Wilkins.

Shanks, J. E., Stelmachowicz, P. G., Beauchaine, K. A., & Schulte, L. (1992). Equivalent ear canal volumes in children pre- and post-tympanostomy tube insertion. *Journal of Speech and Hearing Research, 35*, 936-941.

Shanks, J. E., Wilson, R. H., & Cambron, N. K. (1993). Multiple frequency tympanometry: effects of ear canal volume compensation on static acoustic admittance and estimates of middle ear resonance. *Journal of Speech & Hearing Research, 36*, 178-185.

Shurin, P. A., Pelton, S. I., & Klein, J. O. (1976). Otitis meida in the newborn infant. *Annals of Otology Rhinology and Laryngology, 85 (Suppl)*, 216-222.

Silverman, C. A. (2010). Multifrequency tympanometry in infants and children. *Audiologyonline*, 1-18. www.audiologyonline.com/articles/pf_article_detail.asp?article_id=2341

Sprague, B. H., Wiley, T. L., & Goldstein, R. (1985). Tymapnometric and acoustic reflex studies in neonates. *Journal of Speech and Hearing Reserch, 28*, 265-272.

Stuart, A., Yang, E. Y., & Green, W. B. (1994). Neonatal auditory brainstem response thresholds to air and bone conducted clicks: 0 to 96 hours postpartum. *Journal of American Academy of Audiology, 5*, 163-172.

Sutton, G. J., Gleadle, P., & Rowe, S. (1996). Tympanometry and otoacoustic emissions in a cohort of special care neonates. *British Journal of Audiology, 30*, 9-17.

Swanepoel, D. W., Werner, S., Hugo, R., Louw, B., Owen, R., & Swanepoel, A. (2007). High frequency immitance for neonates: A normative study. *Acta Otolaryngologica, 127*, 49-56.

SWISH. (2011). *NSW Infant screening hearing program (SWISH) - Reminder for newborn hearing tests. Media release by minister for medical research*. Sydney.

Takahara, T., Sando, I., Hashida, Y., & Shibahara, Y. (1986). Mesenchyme remaining in human temporal bones. *Otolaryngology - Head and Neck Surgery, 95*, 349-357.

Taylor, C. L., & Brooks, R. P. (2000). Screening for hearing loss and middle ear disorders in children using TEOAEs. *American Journal of Audiology, 9*, 50-55.

Taylor, P. S., Faeth, I., & Marks, M. K., et al. (2009). Cost of treating otitis media in Australia. *Expert Review Pharmacoeconomics Outcomes Research, 9,* 133-141.

Thornton, A. R. D., Kimm, L., Kennedy, C. R., & Cafarelli-dees, D. (1993). External and middle ear factors affecting evoked otoacoustic emissions in neonates. *British Journal of Audiology, 27*, 319-327.

Tognola, G., Paglialonga, A., & Grandori, F. (2010). Improved newborn hearing screening:Future directions. In C. Driscoll & B. McPherson (Eds.), *Newborn Screening System* (pp. 117-131). San Diego: Plural Publishing.

Toner, J. G., & Mains, B. (1990). Pneumatic otoscopy and tympanometry in the detection of middle ear effusion. *Clinical Otolaryngology and Allied Sciences, 15*, 121-123.

Valvik, B. R., Johnsen, M., & Laukli, E. (1994). Multifrequency Tympanometry. *Audiology, 33*, 245-253.

Van Cauwenberge, P. B., Vinck, B., De Vel, E., & Dhooge, I. (1996). Tympanometry and click evoked otoacoustic emissions in secretory otitis media: are C-EOAEs consistently abset in type B tympanograms? . In L. D.J., C. D. Bluestone, M. Casselbrandt, J. O. Klein, & P. L. Ogra (Eds.), *Recent Advances in Otitis Media* (pp. 139-141). Hamilton, Ontario: B. C Decker.

Vander Werff, K. R., Prieve, B. A., & Georgantas, L. M. (2007). Test-retest reliability of wideband reflectance measures in infants under screening and diagnostic test conditions. *Ear and Hearing, 28*(5), 669-681.

Vanhuyse, V. J., Creten, W. L., & Van Camp, K. J. (1975). On the W-notching of tympanograms. *Scandinavian Audiology, 4*, 45-55.

Vaughan-Jones, R., & Mills, R. P. (1992). The Welch Allyn audioscope and microtymp: Their accuracy and that of pneumatic otoscopy, tympanometry and puretone audiometry as predictors of otitis media with effusion. *Journal of Laryngology and Otology, 106*, 600-602.

Vohr, B. R., Carty, L. M., Moore, P. E., & Letourneau, K. (1998). The Rhode Island hearing assessment program: experience with statewide hearing screening (1993-1996). *Journal of Pediatrics, 133*, 353-357.

Wada, H., & Kobayashi, T. (1990). Dynamical behavior of middle ear: Theoretical study corresponding to measurement results obtained by a newly developed measuring apparatus. *Journal of Acoustical Society of America, 87*, 237-245.

Wada, H., Kobayashi, T., Suetake, M., & Tachizaki, H. (1989). Dynamic behaviour of the middle ear based on sweep frequency tympanometry. *Audiology, 28*, 127-134.

Wada, H., Kobayashi, T., & Tachizaki, H. (1992). Diagnosis of middle ear disease with eardrum perforation by a newly developed sweep frequency measuring apparatus. *Audiology, 31*(3), 132-139.

Wada, H., Koike, T., & Kobayashi, T. (1998). Clinical applicability of the sweep frequency measuring appartus for diagnosis of middle ear diseases. *Ear and Hearing, 19*, 240-249.

Wada, H., Metoki, T., & Kobayashi, T. (1992). Analysis of dynamic behavior of human middle ear using a finite-element method. *Journal of Acoustical Society of America, 92*(6), 3157-3168.

Wallace, I. F., McCarton, C. M., Bernstein, R., Gravel, J. S., Stapells, D., & Ruben, R. J. (1988). Otitis media, auditory sensitivity, and language outcomes at one year. *Laryngoscope, 98*, 64-70.

Wiley, T. L., & Fowler, C. (1997). *Acoustic immitance measures in clinical audiology*. San Diego: Singular Publishing.

Wiley, T. L., Oviat, D. L., & Block, M. G. (1987). Acoustic immittance measures in normal ears. *Journal of Speech Language and Hearing Research, 330*, 161-170.

Wilson, W. (2012). Anatomy and physiology of the outer and middle ear in young infants. In J. Kei & F. Zhao (Eds.), *Assessing Middle Ear Function in Infants* (pp. 1-15). San Diego, CA: Plural.

Yang, E. Y., Stuart, A., Mencher, G. T., Mencher, L. S., & Vincer, M. J. (1993). Auditory brianstem responses to air and bone conducted clicks in the audiological assessment of at-risk infants. *Ear and Hearing, 14*, 175-182.

Young, D. E., TenCate, W.J. F., Ahmad, Z., & Morton, R. P. (2009). The accuracy of otomicroscopy for the diagnosis of paediatric middle ear effusions. *International Journal of Pediatric Otorhinolaryngology, 73*, 825-828.

Zarnoch, J. M., & Balkany, T. J. (1978). Tympanometric screening of normal and ICU borns: Validity and reliability. In E. R. Harford, F. H. Bess, C. D. Bluestone, & J. O. Klein (Eds.), *Impedance screening for middle ear disease in children* (pp. 69-79). New York: Grune and Stratton.

Zhao, F., Wada, H., Koike, T., Ohyama, K., Kawase, T., & Stephens, D. (2002). Middle ear dynamic characteristics in patients with otosclerosis. *Ear and Hearing, 23*(2), 150-158.

Zhao, F., & Wang, J. (2012). Assessing middle ear function in humans using multifrequency tympanometry: An overview. In J. Kei. & F. Zhao. (Eds.), *Assessing middle ear function in infants* (pp. 87-106). San Diego: Plural.

Appendix 1: HREC approvals

Queensland Government

TOWNSVILLE HEALTH SERVICE DISTRICT

Queensland Health
Enquiries to: Cass Donovan
Telephone: 07 4796 1140
Facsimile: 07 4796 1051
Email: cass_donovan@health.qld.gov.au
File Number: SSA/09/QTHS/63
Our Reference: dbs/ethics/RGO/2009/63_1

27 July 2009

Mrs Sreedevi Aithal
Department of Audiology
The Townsville Hospital
IMB 79

Dear Mrs Aithal

HREC reference number: HREC/09/QTHS/30
SSA reference number: SSA/09/QTHS/63
Project title: Identification of middle ear pathology in infants
Protocol number: Protocol Ref N/A

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the following site(s):

The Townsville Hospital

The following conditions apply to this research proposal. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval.

1. As indicated in your protocol submission, this study is scheduled to close on *30/06/2012*. The study cannot continue after this date unless approval has been given to do so. If you require an extension to this date you must send in a written request to us with an explanation of the need for the extension

2. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project are to be submitted to the HREC for review. A copy of the HREC approval/rejection letter must be submitted to the RGO;

3. Proposed amendments to the research protocol or conduct of the research which only affects the ongoing site acceptability of the project, are to be submitted to the research governance officer;

4. A proposed amendment to the research protocol or conduct of the research which may affect both the going ethical acceptability of the project and the site acceptability of the project are to be submitted firstly to the HREC for review and then to the research governance officer after a HREC decision is made.

Should you require any additional information, please do not hesitate in contacting myself on ☎ (07) 47960944.

Yours sincerely

Dr Andrew Johnson
Research Governance Officer
Townsville Health Service District

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

CLINICAL TRIAL
NOTIFICATION SCHEME

Notification of Intent to Supply Unapproved Therapeutic Goods under the Clinical Trial Notification (CTN) Scheme

Therapeutic Goods Act 1989

To be used for:

- **initial notifications of clinical trials involving medicines and/or medical devices under the Clinical Trial Notification (CTN) Scheme; or**
- **notification of one or more additional sites for a Clinical Trial previously reported under the Clinical Trial Notification (CTN) Scheme**

THIS IS THE FORM APPROVED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND AGEING

For detailed information about the CTN Scheme, please see the document *Access to Unapproved Therapeutic Goods - Clinical Trials in Australia* available from the "Unapproved Therapeutic Goods" web page on the TGA Internet site <www.tga.gov.au>.

On completion please send this form to the Therapeutic Goods Administration:

Courier address	*or*	*Postal address*
Financial Services Group		**Financial Services Group**
Therapeutic Goods Administration		**Therapeutic Goods Administration**
136 Narrabundah Lane		**PO Box 100**
Symonston ACT 2609		**Woden ACT 2606**
Australia		**Australia**

Cheques should be made payable to "Therapeutic Goods Administration"

Address: PO Box 100 Woden ACT 2606 Website: www.tga.gov.au
Telephone: 02 6232 8444 Facsimile: 02 6232 8605 ABN 40 939 406 804

PLEASE READ THE FOLLOWING INSTRUCTIONS BEFORE COMPLETING THIS FORM

- Notification under the CTN scheme (or application under the Clinical Trial Exemption (CTX) scheme) is required for clinical investigational use of:
  - any medicine or device not entered in the Australian Register of Therapeutic Goods, including any new formulation of an existing product or any new route of administration; or
  - a marketed medicine or device beyond the conditions of its marketing approval, including new indications extending the use of the product to a new patient group and the extension of doses or duration of treatments outside the approved range.

- **Before completing this form, all parties providing certification should read about their respective responsibilities in the clinical trial.** These roles are outlined in the following documents:
  - Access to Unapproved Therapeutic Goods - Clinical Trials in Australia, TGA, 2004
  - National Statement on Ethical Conduct in Human Research, NHMRC, 2007
  - Guidelines for the Ethical Review of Research Proposals for Human Somatic Cell Gene Therapy and Related Therapies, NHMRC, 1999

- Under the *Therapeutic Goods Act 1989*, the **Therapeutic Goods Administration (TGA) has the authority to enquire into and/or audit clinical trials, where necessary, on safety grounds and to investigate non-compliance with either Good Clinical Practice guidelines or legislative requirements. In addition, information concerning the supply and use of unregistered therapeutic goods may be released to State and Territory regulatory authorities under section 61 of the *Therapeutic Goods Act 1989*.** Completion of this notification form requires the sponsor of the trial, principal investigator, Human Research Ethics Committee and the approving authority to agree, in writing, to make all records available to TGA on request and to cooperate with TGA investigations. The sponsor of the trial is also required to acknowledge the potential for release of information about the use of unregistered therapeutic goods to State and Territory regulatory authorities.

- For the purpose of notifying a Clinical Trial of Medicines or Medical Devices, the **"sponsor of the trial"** is the company, organisation, institution, body or individual (enterprise) that initiates, organises and supports a clinical study of an investigational product on human subjects. As a result, the sponsor of the trial takes responsibility for the overall conduct of the trial. The **"approving authority"** is the body, organisation or institution that approves the conduct of the trial at the site. Thus, the Human Research Ethics Committee (HREC) can also be the Approving Authority for a particular trial site. The same person can sign on behalf of the HREC and the Approving Authority but they should indicate their position or capacity in relation to each. Also, the same person may sign on behalf of the sponsor of the trial and the Approving Authority. However, because of the potential for conflict of interest, the same person cannot sign on behalf of the sponsor of the trial and the HREC.

- **Key points for sponsors of the trial** to check before completing and submitting the CTN form to the Therapeutic Goods Administration (TGA) are:

  - You will need to have a TGA Client ID in order for your notification fee to be accepted and receipted by the TGA Business Management Unit. If you have not conducted business with the TGA before, you will need to obtain a Client ID. Client Details Forms are available from the Experimental Drugs Section or the TGA Business Management Unit and can be submitted simultaneously with this notification.
  - You will need to obtain signatures from the relevant Human Research Ethics Committee, Approving Authority and Principal Investigator for **each** site at which the trial will be conducted. Only ORIGINAL signatures are acceptable.
  - Sites may be notified in any sequence. That is, all sites can be notified in the first instance; notified in groups; or notified singly. The fee for notification of a multi site trial is the same as that for a single site trial providing the sites involved in the multi site trial are declared simultaneously. However, if sites are notified individually or added for an existing trial, an additional fee equivalent to the fee for a single site applies to each notification. Full details of the fee structure for the CTN scheme can be obtained from the Business Management Unit of TGA.
  - Each new and/or additional trial site must be notified to the TGA prior to the trial commencing at that site.
  - You must assign a protocol number to each new trial. Take care not to assign to a new trial a protocol number used previously. Also, check that the protocol number notified to the TGA matches the version of the protocol approved by the Human Research Ethics Committee. When notifying additional sites, quote the protocol number exactly.
  - The TGA assigns a unique clinical trial number. The clinical trial number will appear on an acknowledgement letter from the TGA. Subsequent notifications to TGA of additional trial sites and other correspondence relating to the clinical trial post acknowledgement, such as reporting of adverse reactions, should include the protocol number and the clinical trial number as points of reference.
  - A CTN notification is not effective until the correct fee has been paid.

For office use only

Total Fee Paid	$	Receipt Number	
Client ID Code		TGAIN Number	

SECTION 1. TO BE COMPLETED BY THE SPONSOR OF THE TRIAL

1.1 Notification Type *Complete this section for all notifications. Select one box only. If multiple sites are being notified, complete a 'Trial Site Details' page for each site.*

Initial notification of a single CTN site (new trial) ☑ Subsequent notification of a single additional CTN site ☐

Initial notification of multiple CTN sites (new trial) ☐ Subsequent notification of multiple additional CTN sites ☐

1.2 Potential Use of Restricted Goods *Complete this section for all notifications of medicines only.*

Does this trial involve the use of any medicine as an abortifacient or for "post-coital" or "emergency" contraception in women, or the use of a progesterone antagonist or a vaccine against human chorionic gonadotrophin for any purpose? Yes ☐ No ☐

1.3 Sponsor of the trial *Complete this section for all notifications. In cases where a trial is sponsored by an individual, that person's name may also be the enterprise business name. Business details can be provided to TGA via the Client Details Form. If in doubt, contact the Experimental Drugs Section.*

Sponsor name (Enterprise Business Name): The Townsville Hospital

Client ID Code (If known): 22288

1.4 Trial details

Protocol Number *(Complete for all notifications; maximum of 20 characters)*: 03/2009

If adding a site, Clinical Trial Number *(assigned by TGA; see acknowledgment letter for previously notified sites. Leave blank if unsure)*:

Title of study *Complete for all notifications. Maximum of 255 characters. Title should indicate the aim of the trial and give a broad description of the trial. Include, for example: phase, indication(s) being treated, main medicines and comparators, use of placebo-control, focus of the study, patient population and any other significant or novel aspects. "A Trial of X" is not adequate. Similar detail is required for device trials.*

Identification of middle ear pathology in infants. At present there is no single non invasive test to assess middle ear function in infants. The main aim of this test is to develop middle ear measures including wide band reflectance (WBR), sweep frequency impedance (SFI) and acoustic reflex measures to track the course of middle ear problems in infants and to develop normative data using Wide Band Reflectance Tympanometry and Sweep Frequency Impedance Meter respectively.
A total of 1200 newborn infants over three years who undergo neonatal hearing screening using automated auditory brainstem response audiometry (AABR) will be recruited for this study. Normative data collected using this new devices will be compared with battery of standard audiological tests currently used.

Trial Type ***Complete for initial notification only of trials involving the use of medicines;*** *tick relevant box(es) or otherwise describe.*

Phase 1 ☐ Phase 2 ☐ Phase 3 ☐ Phase 4 ☐ Bioavailability/bioequivalence ☐

Describe if necessary: This trial is use of new devices (WBR Tympanometry and Sweep Frequency Impedance (SFI) meter) to collect normative data to compare with standard tests.

This trial

Complete for initial notification only; tick only those boxes which are applicable. Note: For the purpose of this document, gene therapy includes related therapies that overlap with the traditional concept of gene therapy by virtue of the fact that they introduce DNA into somatic cells. For example, modifications to immunisation strategies in which DNA, rather than protein, is used to generate an immune response for the purposes of prevention or treatment of chronic viral infection or as part of cancer treatment, would be considered a related therapy.

involves the use of a medicine	☐	involves the use of a device	☑
is placebo controlled	☐	is comparator controlled	☐
is also being conducted in other countries	☐	involves gene therapy	☐
Expected trial start date *(Complete for initial notification)*	01/ 06 /2009	**Expected trial completion date** *(Complete for all notifications)*	30/ 06/ 2012

Medicine details

Complete for all notifications of clinical trials involving medicines. Do not use for clinical trials involving the use of devices only. List the therapeutically active components in formulations being used in the trial. All medicines being trialed should be listed, including comparators. The form has space for four medicines. For more than four, attach details of additional medicines in the same format. For the Active Name, enter the active ingredient name using where possible, the Australian Approved Name (AAN). A list of such names (the Approved Terminology for Medicines) is available on the TGA Internet site <www.tga.gov.au> If no AAN, BAN or USAN has been assigned, a code name (see below) or chemical name must be given. For the Code Name, enter code name/s used currently or previously to identify the drug. For the Dosage Form, enter a primary descriptor for dosage form (eg. tablet, injection) and include a secondary descriptor (eg. sustained release, microsphere emulsion) where necessary, particularly if a new dosage form is the focus of the trial.

1	Active name	N/A				
	Trade name		Code name			
	Dosage form		Strength		Biological origin	
2	Active name	N/A				
	Trade name		Code name			
	Dosage form		Strength		Biological origin	
3	Active name	N/A				
	Trade name		Code name			
	Dosage form		Strength		Biological origin	
4	Active name	N/A				
	Trade name		Code name			
	Dosage form		Strength		Biological origin	

Device details

Complete for all notifications of clinical trials involving devices. Do not use for clinical trials involving the use of medicines only. Provide: name (trade name(s), if applicable); description of the device; details of design, composition, specification, mode of action and application; and method of use.

Wide band reflectance (WBR) tympanometry and Sweep Frequency Impedance (SFI) devices will be used to gather data on reflectance of middle ear system, multifrequency tympanograms, ear drum mobility and acoustic stapedius reflexes in infants and children. This will be compared with standard assessments (226 tympanometry, 1000 Hz tympanometry, reflex assessments and otoacoustic emission (OAE) tests).

Device approved name: Wideband Tympanometry Research System
Trade name: Interacoustics Wideband Tympanometry Research System.
Funding has been obtained by statewide Healthy Hearing program for the purchase of above equipment.

Device approved name: Sweep frequency impedance (SFI) meter
Trade name: Sweep frequency impedance meter
This device will be loaned from Audiology department of School of Health and Rehabilitation Sciences, University of Queensland

Both devices are similar to commercially available diagnostic impedance (middle ear analyzer), but use different frequencies, change in pressure variations and signals

An modified commercially available Interacoustics AT 235 Middle ear analyzer with dedicated software and PC is used to collect WBR tympanometry data where as Sweep Frequency Impedance uses its own dedicated system and transfers data to PC.

1.5 Trial site details

Complete for all notifications. Submit a Trial Site Details page for each site at which the trial will be conducted. Enter the name and location of the site (eg. name and address of hospital, institution, clinic or practice). For large institutions, the address need not include specific department details unless essential to identify the location or unless the unit /body/practice operates as a separate entity within the campus. In some rare circumstances, it will be appropriate to notify a trial as a composite site trial. For example, a GP-based trial conducted by a general practice network may need to be notified as a composite site trial. The site details should indicate clearly that there are multiple sites involved and include the name, address and contact numbers for the principal investigator. ***A list of all practices (sites) involved should be submitted as an attachment. The ethics committee and approving authority for such a trial must have appropriate authority for all sites. A sponsor intending to notify a composite site for the first time should contact the Experimental Drugs Section if they have any questions regarding the use of composite sites.***

Site expected start: 06/2009

Site: The Townsville Hospital

Site address: The Audiology Department, IOS, Level 2, Acute Block, IMB 79
100 Angus Smith Road, Douglas, Queensland Post code 4814

1.6 Sponsor certification

Complete this section last for all notifications. In the Name field, print the name of the person signing the form on behalf of the company, organisation, institution, body or individual sponsoring the trial. (Do not enter a company or organisation name here - the entity name appears in Section 1.3) In the Position field, state the person's position within, or relationship to, the entity sponsoring the trial.

I, the undersigned, certify:

- all details contained in this form are true and accurate, and all required information and signatures have been included;
- the sponsor of the trial named in section 1.3 of this form is taking overall responsibility for the conduct of the trial;
- the sponsor of the trial has met or agrees to meet all Human Research Ethics Committee conditions of approval;
- the investigator(s) has/have training and experience relevant to the conduct of this trial;
- the participating institution has resources adequate for the proper conduct of the trial;
- the sponsor of the trial has received an undertaking from the investigator(s) to conduct the trial in accordance with the Guidelines for Good Clinical Practice, as described in regulation 12AB(2)(a) of the Therapeutic Goods Regulations, and the National Statement on Ethical Conduct in Research Involving Humans, as described in regulation 12AD(c) of the Therapeutic Goods Regulations or in regulation 7.3(2a) of the Therapeutic Goods (Medical Devices) Regulations 2002;
- the sponsor of the trial agrees to report all serious and unexpected adverse reactions to the Therapeutic Goods Administration;
- the sponsor of the trial agrees to conduct the trial in accordance with the Guidelines for Good Clinical Practice as described in regulation 12AB(2)(a) of the Therapeutic Goods Regulations and the National Statement on Ethical Conduct in Research Involving Humans as described in regulation 12AD(c) of the Therapeutic Goods Regulations or in regulation 7.3(2a) of the Therapeutic Goods (Medical Devices) Regulations 2002;
- the sponsor of the trial agrees to comply with requests by an authorised officer, whether made before or after the start of a clinical trial, to give information about the conduct of the clinical trial and allow an authorised officer (regulation 2A of the Therapeutic Goods Regulations or regulation 10.1 of the Therapeutic Goods (Medical Devices) Regulations 2002) to do the things mentioned in regulation 12AC and regulation 12AB of the Therapeutic Goods Regulations or in regulation 7.4 of the Therapeutic Goods (Medical Devices) Regulations 2002; and
- the sponsor of the trial accepts that information concerning the use of unregistered therapeutic goods may be released to State and Territory regulatory authorities.

Name (Print)		Position	
Signature	/ /	Phone	
		Fax	

SECTION 2. TO BE COMPLETED BY THE PRINCIPAL INVESTIGATOR

The principal investigator is the person responsible for the conduct of the clinical trial at a trial site. In the case of a trial being conducted by a team of individuals at the site, the principal investigator is the responsible leader of the team.

Principal investigator certification

I, the undersigned:

- am the principal investigator at the site shown in section 1.5 of this form;
- agree to personally supervise the clinical trial at this site in accordance with the relevant current protocol(s) and will only make changes in a protocol after approval by the sponsor;
- have received and read the trial protocol and other relevant information;
- have met or agree to meet all Human Research Ethics Committee conditions of approval for this trial:
- acknowledge my obligations with respect to monitoring patient safety, record management and reporting requirements for adverse events;
- agree to ensure that all associates, colleagues and employees assisting in the conduct of the trial are informed of their obligations in meeting the above requirements;
- agree to promptly report to the Human Research Ethics Committee all unanticipated problems and will not make any changes to the trial without Human Research Ethics Committee and sponsor approval, except where necessary to eliminate apparent immediate hazards to subject safety;
- agree to conduct the clinical trial(s) in accordance with the Guidelines for Good Clinical Practice as described in regulation 12AB(2)(a) of the Therapeutic Goods Regulations and the National Statement on Ethical Conduct in Research Involving Humans as described in regulation 12AD(c) of the Therapeutic Goods Regulations or in regulation 7.3(2a) of the Therapeutic Goods (Medical Devices) Regulations 2002;
- agree to comply with requests by an authorised officer, whether made before or after the start of a clinical trial, to give information about the conduct of the clinical trial and allow an authorised officer (regulation 2A of the Therapeutic Goods Regulations or regulation 10.1 of the Therapeutic Goods (Medical Devices) Regulations 2002) to do the things mentioned in regulation 12AC and regulation 12AB of the Therapeutic Goods Regulations or in regulation 7.4 of the Therapeutic Goods (Medical Devices) Regulations 2002; and
- accept that information concerning the use of unregistered therapeutic goods may be released to State and Territory regulatory authorities.

Name (Print)	Sreedevi Aithal	Phone	(07) 47962763
Signature	Sreedevi 7/5/09	Fax	(07) 47962751

SECTION 3. TO BE COMPLETED BY THE HUMAN RESEARCH ETHICS COMMITTEE RESPONSIBLE FOR MONITORING THE TRIAL

This section must be completed by a Human Research Ethics Committee (HREC) that satisfies the following definition of an ethics committee, as set out in the Therapeutic Goods Act 1989, otherwise the notification is invalid :

> *A committee constituted and operating in accordance with guidelines issued by the National Health and Medical Research Council as in force from time to time and which has notified its existence to the Australian Health Ethics Committee.*

HREC certification should not be given until all conditions of approval of the protocol by that HREC have been met. Wherever possible, the certification should be completed by the Chair or Deputy Chair of the Human Research Ethics Committee. Guidelines for the approval of clinical trials by HRECs are located at 'National Statement on Ethical Conduct in Human Research, NHMRC, 2007' and in the TGA publication 'HRECs and the Therapeutic Goods Legislation'.

For trials of gene therapy and related therapies, the proposal must be approved by all relevant bodies as per the NHMRC Guidelines for Ethical Review of Research Proposals for Human Somatic Cell Gene Therapy and Related Therapies.

HREC name: Townsville Health Service District Human Research Ethics Committee

HREC address: 100 Angus Smith Drive

Douglas Qld Postcode 4810.

Protocol Number approved by HREC:

Does the trial for which approval is being given involve the use of gene therapy or a related therapy? (See NHMRC Guidelines for Ethical Review of Research Proposals for Human Somatic Cell Gene Therapy and Related Therapies) Yes ☐ No ☐

If the trial involves gene therapy or a related therapy, has the Gene and Related Therapies Research Advisory Panel (GTRAP) agreed that the trial can be conducted under the CTN Scheme? Yes ☐ No ☐

Human Research Ethics Committee Certification

I, the undersigned, certify:

- I am a member of the above-named Human Research Ethics Committee;
- the above-named Human Research Ethics Committee is a properly constituted ethics committee and operates in accordance with the guidelines issued by the National Health and Medical Research Council and has notified its existence to the Australian Health Ethics Committee;
- the above-named Human Research Ethics Committee, having regard to the guidance provided by the *National Statement on Ethical Conduct in Research Involving Humans* and, where applicable, the *Guidelines for Ethical Review of Research Proposals for Human Somatic Cell Gene Therapy and Related Therapies*, has approved the clinical trial protocol identified above and has assumed responsibility for monitoring the conduct of the trial; and
- the above-named Human Research Ethics Committee agrees to comply with requests by an authorised officer, whether made before or after the start of a clinical trial, to give information about the conduct of the clinical trial and allow an authorised officer (regulation 2A of the Therapeutic Goods Regulations or regulation 10.1 of the Therapeutic Goods (Medical Devices) Regulations 2002) to do the things mentioned in regulation 12AC and regulation 12AB of the Therapeutic Goods Regulations or in regulation 7.4 of the Therapeutic Goods (Medical Devices) Regulations 2002.

Name (Print): DR ISAAC SEIDL

Position: Chair HREC

Signature: 21/7/09

Phone:

Fax:

SECTION 4. TO BE COMPLETED BY THE AUTHORITY APPROVING THE CONDUCT OF THE TRIAL

Complete for all notifications. In cases where the Human Research Ethics Committee or Approving Authority for more than one site is the same, it is still necessary to submit a Trial Site Details Page for ***each*** *site. The bodies approving the conduct of the trial at each site need to be declared individually. This requirement also still applies in cases where, for example, an Area Health Service or Hospitals Group may encompass several different institutions.*

The Approving Authority must appoint a person to be responsible for giving approval on its behalf. The terms of approval for the conduct of the trial must be consistent with the Human Research Ethics Committee's (HREC) recommendations and these terms must be no less restrictive than the HREC advice.

Approving Authority name	Townsville Health Service District - The Townsville Hospital
Address	100 Angus Smith Drive
	Douglas Qld Postcode 4810

Approving Authority Certification

I, the undersigned

- am authorised to represent the body, organisation or institution at which the above mentioned clinical trial will be conducted and, having regard to the advice and approval of the trial protocol by the Human Research Ethics Committee responsible for monitoring the trial at this site, give approval for this trial to proceed;
- undertake that the use of the drug will comply with all relevant Commonwealth and State or Territory legislation; and
- undertake to comply with requests by an authorised officer, whether made before or after the start of a clinical trial, to give information about the conduct of the clinical trial and allow an authorised officer (regulation 2A of the Therapeutic Goods Regulations or regulation 10.1 of the Therapeutic Goods (Medical Devices) Regulations 2002) to do the things mentioned in regulation 12AC and regulation 12AB of the Therapeutic Goods Regulations or in regulation 7.4 of the Therapeutic Goods (Medical Devices) Regulations 2002.

Name (Print)	Mary Bone	Position	DCEO
Signature	M Bone 7,7,09	Phone	
		Fax	

THE UNIVERSITY OF QUEENSLAND

Institutional Approval Form For Experiments On Humans Including Behavioural Research

Chief Investigator:	Mrs Sreedevi Aithal, Mr Venkatesh Aithal
Project Title:	Identification Of Middle Ear Pathology In Infants
Supervisor:	Dr Joseph Kei, Dr Carlie Driscoll
Co-Investigator(s)	Dr Joseph Kei, Dr Andrew Swanston, Katrina Roberts
Department(s):	Division of Audiology, School of Health and Rehabilitation Sciences
Project Number:	2010000842
Granting Agency/Degree:	QLD Health
Duration:	31st July 2013

Comments:

Expedited review on the basis of approval from the Townsville HSD HREC, dated 27/07/2009.

Name of responsible Committee:-
Behavioural & Social Sciences Ethical Review Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Dr Jack Broerse
Chairperson
Behavioural & Social Sciences Ethical Review Committee

Date 06/07/10 Signature

Queensland Government

Queensland Health

TOWNSVILLE HEALTH SERVICE DISTRICT

Enquiries to: Medical Administration
Telephone: 07 4796 1140
Facsimile: 07 4796 1051
Email: TSV-Ethics-Committee@health.qld.gov.au
Our Reference:
dbs/ethics/Protocol/2012/Correspondence

16 March 2012

Mrs Sreedevi Aithal
Department of Audiology
The Townsville Hospital
IMB 79

Dear Mrs Aithal

HREC reference number: HREC/09/QTHS/30
Project title: Identification of middle ear pathology in infants
Protocol number:

Thank you for your letter dated 13 February 2012. The above amendment was reviewed by the Chair of the Townsville Health Service District Human Research Ethics Committee. Your extension request to 31 December 2013 has been approved.

The Townsville Human Recourse Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's *"National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *"CPMP/ICH Note for Guidance on Good Clinical Practice"*.

A copy of this letter must be forwarded to the Townsville Health Service District Research Governance Officer.

It should be noted that all requirements of the original approval still apply.

Should you require any additional information, please contact me on (07) 4796 1140.

Yours sincerely

K S Sangla

Dr Kunwarjit Sangla
Chairperson
Townsville Health Service District
Human Research Ethics Committee

09/QTHS/30_7
Human Research Ethics Committee
Medical Services Support Unit
07 4433 1140

Queensland Government

Townsville
Hospital and Health Service

3rd May 2013

Sreedevi Aithal
Audiology Department
The Townsville Hospital
IMB 79

Dear Sreedevi,

HREC Reference number: HREC/09/QTHS/30
Project title: Identification of middle ear pathology in infants

Thank you for submitting an amendment for the above mentioned study on 01/05/2013. The correspondence was reviewed at the meeting of the Chairperson held on 03/05/2013.

I am pleased to advise that the amended documents reviewed and approved at the meeting were:

Document	Version	Date
Notification of amendment – *Request for extension*		22.04.13

HREC Approval is now valid until 31/12/2016.

The Annual Report included in the submission dated 22/04/2013 was also reviewed and noted by the Townsville Hospital and Health Service Human Research Ethics Committee Chair on 03/05/2013.

The Townsville Hospital and Health Service HREC is constituted and operates in accordance with the National Health and Medical Research Council's "*National Statement on Ethical Conduct in Human Research (2007), NHMRC* and *Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *"CPMP/ICH Note for Guidance on Good Clinical Practice"*.

A copy of this letter must be forwarded to the Townsville Hospital and Health Service Research Governance Office/r.

It should be noted that all requirements of the original approval still apply.

Yours sincerely,

A/Prof Andrew Johnson
Chairperson
Townsville Hospital and Health Service
Human Research Ethics Committee

Townsville Hospital and Health Service
Human Research Ethics Committee
Telephone +617 4433 1140
Email TSV-Ethics-Committee@health.qld.gov.au

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	Identification Of Middle Ear Pathology In Infants - 16/04/2013 - AMENDMENT
Chief Investigator:	Sreedevi Aithal, Venkatesh Aithal
Supervisor:	Dr Joseph Kei, Dr Carlie Driscoll
Co-Investigator(s):	Dr Joseph Kei, Dr Andrew Swanston, Katrina Roberts
School(s):	Division of Audiology, School of Health and Rehabilitation Sciences
Approval Number:	2010000842
Granting Agency/Degree:	NHMRC
Duration:	31st December 2016

Comments:

Note: If this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
Behavioural & Social Sciences Ethical Review Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Associate Professor John McLean
Chairperson
Behavioural & Social Sciences Ethical Review Committee

Signature _____ Date 16/4/2013

Appendix 2: Parent information sheet and consent form

Queensland Government
Queensland Health

Audiology Department
Townsville Health Service District

Level 2 Acute Block
Surgical Clinics
The Townsville Hospital
PO Box 670 ,Townsville Q 4810

Telephone: 07 4796 2765
Facsimile: 07 4796 2810

PARENT /CAREGIVER /PARTICIPANT INFORMATION SHEET

Project Title: Identification of middle ear pathology in infants

Investigators:
Sreedevi Aithal, MSc, MPH, Consultant Paediatric Audiologist, The Townsville Hospital.
Venkatesh Aithal, MSc, MPH, Consultant Audiologist, The Townsville Hospital
Dr. Andrew Swanston, FRCS, FRACS, Director of ENT Services & Associate Professor, JCU Medical School, The Townsville Hospital
Dr Joseph Kei, PhD, Head, Division of Audiology, University of Queensland
Katrina Roberts, Nursing Director, Health and Wellbeing Service group, Townsville Hospital

Ethical clearance:
This study has been reviewed and approved by the Townsville Health Service District Human Research Ethics Committee. Should you wish to discuss the study with someone not directly involved, particularly in relation to matters concerning policies, information about the conduct of the study or your rights as a participant; or should you wish to make an independent complaint you can contact the Chairperson, Townsville Health Service District Human Research Ethics Committee, PO Box: 670, Townsville, Qld 4810, Telephone (07) 47961140.

Research Aim:
Babies with middle ear problems are at risk of a speech and language delay and related learning problems later in childhood. Therefore it is important to identify problems early in life, so they can be managed appropriately. This study will look into the measurement of ear drum and middle ear mobility, movement and absorbance of different frequency sounds as an alternative new technique of diagnosing the middle ear problems in babies.

Procedure:
Two new tests (described below) will be administered to identify middle ear problems in babies and each test will take about 10 to 15 minutes. This result will be compared with standard test results. Neither test will cause discomfort to the baby except presentation of sounds with different tones to the ear canal with changing pressure in the ear canal similar to the one administered during standard routine audiological tests to the babies.

Testing will be performed in your presence and testing time will fit in with your schedule. We shall also try to follow up your baby's ears again 1, 2, 4,6,12 and 24 months along with

immunisation schedule in order to understand the longitudinal history of the middle ear problems. During follow up period, we will provide you with questionnaire at 12 and 24 months to check the history of recurrent ear infections and risk factors.

Following new tests will be conducted.

1. Wideband Reflectance (WBR) tympanometry test involves measurement of baby's ear drum and middle ear movements (testing middle ear function). This test involves placing a small plastic tube in the ear canal and presenting sound in 226 Hz to 8 KHz frequency range and measuring absorbance of sound at varying pressure and frequency levels in the ear canal. A series of sound of different pitch will also be presented to the ear to check for middle ear muscle contraction response.
2. Sweep Frequency Impedance (SFI) test involves placing a small plastic tube known as probe in the ear canal and presenting sound varying in frequency from 0.2 to 2.0 kHz with changing pressure in the ear canal. The sound pressure variation in the ear canal is measured at different frequencies and is recorded. This test also measures the displacement of ear drum at different air pressure levels in the ear canal.

Equipment:
The equipment for WBR test is provided by State Wide Healthy Hearing Program which introduced Universal Newborn Hearing Screening (UNHS) across the state and SFI equipment by Audiology department of University of Queensland.

The result of above test will be compared with standard tests (1000 Hz tympanometry, acoustic reflexes for tone and wideband noise and otoacoustic emission (TEOAE) test).

A "PASS" in standard test means that your baby's ears are working normal at the time of testing. But it does not necessarily mean normal for life. That is why it is important to follow up regularly. If, however, you have any doubts about your child's hearing later, you should contact your family doctor to arrange a hearing test for your child. We shall follow up your child's ears during his/her immunisation schedule.

A "REFER" in standard test means your child may have some hearing and middle ear problems. In this case we will arrange the full diagnostic tests immediately. This could be due to several reasons such as debris still trapped in the ear canal (a common problem with babies), noisy test room, unsettled baby during test etc.

Possible risks
The tests are not invasive and will not cause any discomfort to babies. There are no reported adverse affects of these tests.

Potential benefits of the study
The benefits of this study are free checks for middle ear problems for babies born at The Townsville Hospital and longitudinal monitoring of their middle ear condition. A specialist referral will be made when indicated. The data derived from this study will be used in Healthy Hearing programs currently funded by Queensland Health.

Confidentiality of test results
You are assured that no information regarding your baby's results will be divulged and the results of any test will not be published so as to reveal your baby's identify.

Withdrawal from the study
Your participation is entirely voluntary. You can withdraw your child from the project at any stage of the study. This will not affect in a way the provision of healthcare, now or in the future for your child.

Thank you for your interest in this project.

Queensland Government
Queensland Health

Audiology Department
Townsville Health Service District

Level 2 Acute Block
Surgical Clinics
The Townsville Hospital
PO Box 670 ,Townsville Q 4810

Telephone: 07 4796 2765
Facsimile: 07 4796 2810

PARENT CONSENT FORM

Project Title: Identification of middle ear pathology in infants

Investigators:
Sreedevi Aithal, MSc, MPH, Consultant Paediatric Audiologist, The Townsville Hospital.
Venkatesh Aithal, MSc, MPH, Consultant Audiologist, The Townsville Hospital
Dr. Andrew Swanston, FRCS, FRACS, Director of ENT Services & Associate Professor, JCU Medical School, The Townsville Hospital
Dr Joseph Kei, PhD, Head, Division of Audiology, University of Queensland
Katrina Roberts, Nursing Director, Health and Wellbeing Service group, Townsville Hospital

1. The nature and purpose of the research project has been explained to me. I understand it, and agree to take part.
2. I have been given an information sheet which explains the purpose of the study, the possible benefits and possible risks.
3. I understand that child may not directly benefit from taking part in this study
4. I understand that, while information gained during the study may be published, child will not be identified and his/her personal results will remain confidential
5. I understand that I can withdraw my child from the study at any stage and that it will not affect his/her medical care, now or in the future.
6. I understand that there is no payment is made to me or child for taking part in this study which is explained in the information sheet.
7. I have had the opportunity to discuss taking part in this investigation with a family member or friend.

Name of the child: _____DOB:_____
(Please affix the label)

Parental / Caregiver signature: _____Date:_____
Telephone No:
Address:

I certify that I have explained the study to the parent and consider that he/she understands what is involved.

Signature:_____Date: _____
(Signature of person involved in the research) Research officer/Nurse/Investigator

Signed:_____Date:_____

(Witness)