WIDEBAND ENERGY REFLECTANCE MEASUREMENTS: NORMATIVE STUDY AND EFFECTS OF NEGATIVE AND COMPENSATED MIDDLE EAR PRESSURES

A Dissertation by

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Bachelor of Arts, Wichita State University, 2004

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WIDEBAND ENERGY REFLECTANCE MEASUREMENTS: NORMATIVE STUDY AND EFFECTS OF NEGATIVE AND COMPENSATED MIDDLE EAR PRESSURES

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DEDICATION

For my mother, my wife, and my children
ABSTRACT

Conventional clinical procedures for middle ear assessment have been used for several decades but have shown limitations. Application of a new technology, wideband energy reflectance (ER), has shown great potential. The ER measurement determines the proportion of acoustic energy reflected by the middle ear, across a broad frequency range. Negative middle ear pressure (MEP) is a highly prevalent, and mostly transient, form of middle ear dysfunction which effects ER measurement. Goals of the present study were to examine various factors relating to the ER test: (1) test-retest reliability, (2) the effects of pressure manipulations, (3) the effects of negative MEP, and (4) the effectiveness of a corresponding compensation procedure. Data were collected in 48 adults and analyzed across the frequency range from 0.223 to 8 kHz. Measurements were conducted using both ambient and dynamic pressure methods, under three conditions: normal MEP, negative MEP, and compensated negative MEP.

Correlation between immediately repeated tests were strong for all frequencies. The sweeping pressure procedure caused ER reduction for a few frequencies but differences were small. Thirty-five subjects were able to produce a negative MEP ranging from -40 to -220 daPa. Negative MEP increased ER at low- and mid-frequencies while decreasing ER at high-frequencies. Magnitude of changes and frequency at which maximum change occurred increased when MEP became more negative. Compensated negative MEP reduced ER at low- and mid-frequencies but increased ER at high-frequencies. The present study demonstrated that negative MEP altered ER in a frequency-specific pattern. The compensation procedure eliminated the effects of negative MEP. Immediate test-retest reliability of the ambient ER test was excellent. Both probe reinsertion and manipulation of the ear canal and middle ear pressure produced minimal effects.
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Sound transmission through the auditory system is dependent on the sound transmission characteristics of the middle ear. Alterations in the stiffness and mass components of the middle ear system decrease the efficiency of sound transmission resulting in reduced audibility. A common example would be the increased middle ear stiffness resulting from a negative pressure gradient across the tympanic membrane. If the Eustachian tube becomes dysfunctional, middle ear pressure gradually becomes negative relative to ambient pressure. This pressure differential increases middle ear stiffness resulting in a greater impedance mismatch between the air within the ear canal and the fluids within the inner ear. It is important to clarify the nature of these effects for both basic science and clinical researchers to develop a better understanding of middle ear function. Conventional tests of middle ear function (i.e., single low-frequency tympanometry) provide little information regarding the sound transmission characteristics of the middle ear system. Further, diagnostic performance of conventional tests have been called into question.

Previous studies utilizing wideband energy reflectance, a new test of middle ear function, have demonstrated the ability to characterize the sound transmission characteristics of the middle ear as well as improve diagnostic performance when assessing various middle ear pathologies. The effects of negative middle ear pressure on the wideband energy reflectance test are of particular interest due to its high prevalence. Recent studies reporting the effects of negative middle ear pressure in clinical populations are limited by methodological design and diagnostic
uncertainty. Results from observational designs cannot establish a causal relationship because the independent variable, middle ear pressure, is not under experimenter control. Moreover, current methods of clinical assessment in ears with negative MEP cannot rule out the presence of middle ear fluid.

A need exists to establish the effects of negative MEP on wideband energy reflectance measurements under laboratory conditions, where middle ear pressure can be manipulated without uncertainty regarding the presence of middle ear fluid. Previous research has demonstrated that the effects of negative MEP on tests of middle and inner ear function can be compensated for so that tests may remain sensitive to co-existing middle ear or underlying inner ear pathologies. The effectiveness of this compensation has not been systematically investigated using the wideband energy reflectance technique.
CHAPTER 2
LITERATURE REVIEW

Structure of the Human Ear

The human ear may be divided into three units based on structural and functional differences: external, middle, and inner ear. The former two units play an integral role in sound conduction to the inner-ear where mechanical energy is transduced into neurochemical signals which travel along the auditory nerve to the brain. The external ear consists of the pinna and the ear canal. Pinnae are attached to each side of the head and are comprised of both skin-covered cartilage and fat with various grooves and ridges (Goode, 2001). The 6 to 7 mm ear canal aperture allows sound waves to propagate along its 25 mm length (Goode, 2001; Møller, 2000). The medial boundary for the “S”-shaped ear canal occurs at the tympanic membrane (eardrum).

Sound waves propagate down the external auditory canal and set the thin, conical tympanic membrane into motion (Yost, 2000). The tri-layered tympanic membrane has a slight concavity in its orientation with a diameter of around 10 mm and a surface area of around 85 mm$^2$ (Goode, 2001; Keefe & Feeney, 2009; Møller, 2000). An anatomical distinction is made between the thicker, superior portion of the tympanic membrane, the pars flaccida, and the thinner pars tensa which comprises 65% of its total area. It has been found that only the pars tensa contributes to tympanic membrane motion at high sound pressure levels (Yost, 2000).

On the medial side of the tympanic membrane is the middle ear cavity, or tympanum (Møller, 2000). It houses the ossicular chain (comprised of three tiny bones: malleus, incus, and stapes) which provides a bridge between the tympanic membrane and the inner ear. The ossicles
are suspended by ligaments with the manubrium of the malleus embedded into the tympanic membrane and its head forming a rigid, double saddle joint with the incus inside the epitympanic recess. Medially, the lenticular process of the incus articulates with the stapes at the incudostapedial joint. Two muscles are present within the middle ear cavity: the tensor tympani which inserts onto the manubrium of the malleus and the stapedius which attaches to the stapes.

Communication between the middle ear and the nasopharynx is provided by the one-way valvular action of the Eustachian tube (Møller, 2000; Yost, 2000). This Eustachian tube provides a pressure equalization function crucial for maintaining middle ear pressure near ambient pressure (Yost, 2000). Without this function, changes in air pressure (e.g., due to changes in elevation) would produce either a compressed or taut ossicular chain resulting in a decrease in the efficiency of sound transmission.

Medial to the footplate of the stapes, resting within the oval window, is the cochlea. It is a coiled structure which contains slightly more than 2½ turns (Møller, 2000). Within the cochlea lies the organ of Corti, the sensory end organ of hearing. Its sensory receptors are called hair cells and are arranged in rows along the basilar membrane. Afferent and efferent neurons synapse with these hair cells for purposes of both signal transmission to and biofeedback from the central auditory nervous system.

Function of the Human External and Middle Ear

Sound waves that propagate through the environment are altered by the pinna, the ear canal, and the head in a frequency-specific manner (Møller, 2000). The ear canal acts as a
quarter-wave resonator due to its medial boundary at the tympanic membrane and its open end at the entrance to the ear canal. Based on the length of the average human ear canal, a natural resonance around 3 kHz results in the sound pressure at the tympanic membrane being about 10 dB higher at the resonance frequency relative to the sound pressure at the ear canal entrance. When the filtering effects of the pinna, ear canal, and head are combined, sound presented in front of the head (i.e., 0° azimuth) and measured at the tympanic membrane is around 15 dB greater between 2 and 4 kHz compared to sound pressure measured at the ear canal entrance.

Sound waves impinging on the tympanic membrane cause it to vibrate, setting the ossicular chain into motion, and creating a traveling wave on the basilar membrane within the cochlea (Keefe & Feeney, 2009; Møller, 2000; Yost, 2000). This middle ear mechanism, acting as a bridge between the ear canal and the cochlea, is crucial to normal auditory function. If sound waves were to be applied to cochlear fluids directly, only 0.1% of sound energy would be absorbed due to the large differences in density between air and fluid; this results in a large impedance mismatch between the air in the middle ear and the cochlear fluids of the inner-ear.

Impedance (Z) refers to the ratio of an applied force to the volume velocity (U) resulting from the applied force (Haughton, 2002; Margolis & Hunter, 1999). When referring to acoustic impedance, the force may be described in terms of sound pressure (P) (Rosowski & Relkin, 2001).

\[ Z = \frac{P}{U} \]  

(1.1)

The lower the velocity achieved by a given force or sound pressure, the greater the impedance and opposition to the flow of energy. Since the sinusoidal variation of P and U may not coincide
in time, useful information may be derived from calculating the phase angle ($\Theta$) of $Z$ which refers to the algebraic phase difference (Rosowski & Relkin, 2001).

$$\Theta = \text{phase of } P - \text{phase of } U$$ (1.2)

Impedance is further described as consisting of two components: resistance ($R$) and reactance ($X$). Resistance is the real part of impedance and may be defined as the ratio between the sound pressure and the in-phase component of volume velocity (Haughton, 2002). Resistive forces at the tympanic membrane include viscous damping in addition to absorption of sound by the inner ear (Rosowski & Relkin, 2001). Resistance equals acoustic impedance when sound pressure and volume velocity are in-phase ($\Theta = 0^\circ$), resulting in the system's maximum response. Reactance is the imaginary part of impedance and may be defined as the ratio between the sound pressure and the leading/lagging component of volume velocity (Haughton, 2002). Reactive components store energy for up to one-quarter cycle. In instances where these phase differences between sound pressure and volume velocity are greatest (i.e., $\Theta = +/- 90^\circ$), acoustic impedance increases resulting in reduced sound transmission through the middle ear system.

The reactive component of acoustic impedance may be subdivided into mass reactance ($X_m$) and stiffness reactance ($X_r$) (Rosowski & Relkin, 2001). At low-frequencies volume velocity leads sound pressure by up to one-quarter cycle ($\Theta = -90^\circ$). This phase lead indicates that the middle ear system is dominated by the stiffness of the tympanic membrane, ossicular ligaments, and air within the middle ear cavity. At resonance where there is no phase difference between volume velocity and sound pressure ($\Theta = 0^\circ$), the middle ear system is dominated by resistance. At high-frequencies where volume velocity lags behind sound pressure by up to one-quarter
cycle ($\Theta = +90^\circ$), the middle ear system is dominated by the mass of the tympanic membrane, ossicular chain, and cochlear fluids.

The crucial function of the tympanic membrane and ossicular chain is to form an impedance-matching bridge between the air in the external ear and the oval window membrane leading to the inner ear. Since the tympanic membrane has a relatively low impedance, but the oval window (leading to the cochlear fluids of the inner ear) has a relatively high impedance, adding an impedance-matching transformer maximizes the efficiency of energy transmission. The ossicular chain creates this bridge between the tympanic membrane and the cochlea.

To maximize sound transmission, impedance differences need to be reduced (Austin, 1994). In the middle ear system this is accomplished by three mechanisms comprising the middle ear transformer: (a) the catenary lever, (b) the ossicular lever, and (c) the hydraulic lever. The catenary principle states that when a fixed sound pressure is applied, the pressure at the outer portions of the tympanic membrane will be transferred to the apex where the manubrium of the malleus is embedded (Austin, 1994; Musiek & Baran, 2007). These effects have been thought to be due to the immobility of the bony annulus. This buckling action that occurs during tympanic membrane vibration results in a doubling of force transmitted to the ossicular chain. Due to the increased length of the manubrium and neck of the malleus relative to the long process of the incus, force is further amplified by a factor of 1.3 and attributed to the action of the ossicular lever (Austin, 1994; Yost, 2000). However, it is the hydraulic lever which provides the greatest gain advantage. Due to the areal ratio between the tympanic membrane and the stapes footplate (area of the stapes footplate is 17 to 20 times smaller than the effective area of the tympanic
membrane), force at the stapes footplate is increased by 17 to 20x resulting in a gain of 25 dB or more. However, it is important to realize that the gain created by the middle ear impedance-matching transformer is frequency dependent.

Pathologies of the Middle Ear

Various middle ear pathologies affect sound transmission to the inner ear by altering the mass and/or stiffness of the middle ear system. This alters the middle ear input impedance resulting in less efficient sound transmission to the inner ear and hearing loss. When stiffness is reduced by a perforation in the tympanic membrane or a discontinuity in the ossicular chain, a low impedance middle ear pathology is the result. When stiffness increases, as in otosclerosis, negative middle ear pressure, or otitis media, a high impedance middle ear pathology is produced.

Low Impedance Pathologies of the Middle Ear

Low impedance pathologies refer to various forms of middle ear dysfunction which lower the impedance of the middle ear system relative to the normal middle ear system. (Margolis & Hunter, 1999). Most often these changes occur at the site of the tympanic membrane where its integrity is compromised due to rupture or membrane atrophy. Less commonly, the site-of-lesion may occur more medially where disarticulation or erosion of either ossicular joint decreases middle ear system stiffness. These low impedance pathologies are less prevalent than their high impedance counterparts. Clinical examples include ears with tympanic membrane perforations or
ossicular disruptions.

**Tympanic Membrane Perforations**

Perforations of the tympanic membrane often occur secondary to middle ear disease, either from the disease process or through treatment by placement of tympanostomy tubes. They may also result from trauma such as blast injuries, use of cotton swabs, diving, as well as other routes of injury. The age-adjusted prevalence of chronic tympanic membrane perforation is around 0.45% (Kaftan, Noack, Friedrich, Völzke, & Hosemann, 2008).

Measurements of sound pressure level and stapes velocity, via laser Doppler vibrometry (LDV), in human cadaver temporal bones have revealed that tympanic membrane perforations of all sizes have their greatest effects on sound transmission at low frequencies (Voss, Rosowski, Merchant, & Peake, 2001a). These effects decrease with increasing frequency up to 1 to 2 kHz. As perforation size increases, both transmission loss and clinically-measured hearing loss increase (Austin, 1978; Ibekwe, Nwaorgu, & Ijaduola, 2009; Voss et al., 2001a, 2001b). At low-frequencies this loss increases monotonically and has a slope of around 40 dB per decade. At mid-frequencies, between 1 and 2 kHz, there is either no loss or even enhancement as high as 20 dB. The frequency at which this enhancement occurs increases with increasing perforation size (Voss et al., 2001a, 2001b). For frequencies above 2 kHz, transmission losses do occur, but they typically do not exceed 10 dB. A similar pattern of transmission loss has been demonstrated in cochlear potential recordings in cat (Kruger & Tonndorf, 1977). Larger middle ear volumes reduce the magnitude of this effect (Ahmad & Ramani, 1979; Voss et al., 2001a, 2001b, 2001c).
Inter-subject variability in middle ear volumes may explain why mid-frequency threshold enhancements have not been reported in clinical studies.

Austin (1978) proposed that hearing loss due to tympanic membrane perforations may be attributed to areal ratio loss. More generally, this would lead to altered coupling of the pressure difference across the tympanic membrane to the motion of the malleus (Voss et al., 2001a). Alternatively, the transmission alterations may be ascribed strictly to the altered pressure difference across the tympanic membrane. Sound pressure level and LDV measurements made in human temporal bones provide strong support for the latter mechanism.

**Ossicular Discontinuity**

Trauma or disease processes may result in interruption of the ossicular chain (Møller, 2000). Disarticulation of the incudostapedial joint is often due to problems with a surgical prosthesis or erosion of the long process of the incus (Austin, 1994). Ossicular discontinuities occurring secondary to chronic otitis media have been reported to occur in approximately 30 to 45% of patients undergoing their first otologic surgery (Carrillo, Yang, & Abes, 2007; Jeng, Tsai, & Brown, 2003). Ears with discontinuities may present with various audiometric configurations and considerable variability (Rosowski, Nakajima, & Merchant, 2008). This variability may be, at least partially, attributed to inter-individual differences in the nature of the discontinuity (e.g., partial vs. total interruptions).

Non-invasive LDV has been utilized to measure umbo velocity in ears with surgically-confirmed ossicular discontinuity (Huber et al., 2001; Jakob, Bornitz, Kuhlisch, & Zahnert,
Mean umbo velocity has been shown to be significantly higher in these ears below 1 kHz (Rosowski et al., 2008), whereas peak umbo displacement (derived from velocity measurements) has been shown to be higher below 1.5 kHz (Huber et al., 2001). The largest increase in velocity was on the order of 16 dB and occurred around 0.7 kHz (Rosowski et al., 2008). Umbo velocity and displacement tend to fall within the normal range at mid-frequencies but decrease at higher frequencies with displacement significantly lower than normal above 5.5 kHz (Huber et al., 2001). Mean phase angles derived from umbo velocity measurements were also significantly reduced between 0.5 and 4 kHz (Rosowski et al., 2008). Collectively, these results are consistent with decreased stiffness in the middle ear systems of ears with ossicular interruptions.

High Impedance Pathologies of the Middle Ear

High impedance pathologies of the middle ear decrease the admittance of sound energy through increased stiffness and/or increased mass of the middle ear system. Frequently occurring examples of high-impedance pathologies include otosclerosis, otitis media, and negative middle ear pressure.

Otosclerosis

Decreased sound transmittance through the middle ear due to fixation of the stapes footplate in the oval window occurs in a condition called otosclerosis (Møller, 2000). In an epidemiological study conducted in Britain, the prevalence rate for a presumptive diagnosis of
otosclerosis was determined to be 2.1% (Browning & Gatehouse, 1992). The disease is progressive with hearing adversely affected in the low-frequencies more than in the high frequencies. In later stages of the disease, low-frequency thresholds are around 50 dB HL with higher frequencies less affected.

Several investigators have used LDV to measure the mobility of the ossicular chain in both human temporal bone preparations and, non-invasively, in ears with surgically-confirmed otosclerosis. Peak umbo displacement, as well as both umbo displacement and umbo velocity transfer functions, have been reported to be significantly lower in otosclerotic ears (re: normal ears) for frequencies below 1 kHz (Huber et al., 2001; Jakob et al., 2009; Rosowski et al., 2008). Reduction for umbo displacement and umbo velocity transfer functions is on the order of 6 dB. Peak umbo displacement and umbo displacement transfer functions have been shown to be higher for frequencies between 1.5 and 2.8 kHz and higher by around 3.5 dB for frequencies above 1.8 kHz, respectively (Huber et al., 2001; Jakob et al., 2009). Significantly larger magnitudes for high frequencies were present around 3 and 4 kHz for umbo velocity transfer functions (Rosowski et al., 2008). Phase angle of the umbo velocity transfer function was also slightly larger below 1.5 to 3 kHz (Rosowski et al., 2003; Rosowski et al., 2008). Further, the first dominant resonance taken from peak umbo velocity measurements was significantly higher in otosclerotic ears compared to normal ears (Huber et al., 2001).

*Otitis Media with Effusion*

Otitis media with effusion (OME) refers to the presence of middle ear fluid in the
absence of infection (American Academy of Pediatrics, 2004). By 1-year of age, more than half of all children will have experienced OME with rates increasing to greater than 60% by 2-years and greater than 90% by 3-years of age (American Academy of Pediatrics, 2004; Casselbrant & Mandel, 1999; Møller, 2000). The point prevalence varies widely across countries and is likely influenced by differences in study methodology. (Casselbrant & Mandel, 1999).

The presence of effusion within the middle ear cavity reduces tympanic membrane compliance by decreasing middle ear volume and increasing tympanic membrane mass (Ravicz, Rosowski, & Merchant, 2004). Effects of middle ear fluid on middle ear mechanics have been analyzed by measuring umbo velocity via LDV measurements in human temporal bones (Dai, Wood, & Gan, 2007; Dai, Wood, & Gan, 2008; Gan, Dai & Wood, 2006; Ravicz et al., 2004).

Use of a human temporal bone model allows for greater experimental control without creating ethical concerns. LDV has been used to demonstrate changes in normalized umbo velocity and peak-to-peak umbo displacement when saline was introduced into the middle ear cavity to simulate serous effusion (Dai et al., 2007; Dai et al., 2008; Gan et al., 2006; Ravicz et al., 2004). Measurements of peak-to-peak umbo displacement suggest that there is a critical volume at which tympanic membrane displacement is reduced (Dai et al., 2008; Gan et al., 2006). In human temporal bones this was found to be around 0.3 ml, or half of the middle ear volume. At this threshold level, displacement at frequencies of 1 kHz and higher was reduced. This reduction amounted to 6 dB at 1 kHz and 10.5 dB at 6 kHz (Gan et al., 2006). Increasing amounts of fluid resulted in greater reductions in umbo displacement, especially at frequencies greater than 0.3 kHz. Complete filling of the middle ear (0.6 ml) resulted in around 20 dB
attenuation between 1 and 6 kHz (Dai et al., 2007; Gan et al., 2006).

When the amount of saline needed to cover 50% of the tympanic membrane and reduce middle ear volume by 30% was infused into the middle ear space, it caused normalized umbo velocity to be reduced by around 2 dB at 0.4 kHz, unchanged or slightly increased at 0.7 kHz, and attenuated by 10 to 12 dB at 3 kHz (Ravicz et al., 2004). Phase angle was reduced above 0.4 kHz with a negative phase shift of around 55° above 1 kHz. Additional saline was added to completely cover the tympanic membrane and reduce middle ear volume by 60%; normalized umbo velocity above 0.7 kHz was reduced from 20 dB to greater than 30 dB. Further saline was added reducing middle ear volume by 90%. Low-frequencies were most affected with a 6 to 8 dB reduction occurring below 0.15 kHz.

Low-frequency (< 0.8 kHz) reductions were dependent on the quantity of middle ear fluid but the percentage of the tympanic membrane in contact with the fluid was not a factor (Ravicz et al., 2004). This suggests that reductions in middle ear volume were responsible for changes in this stiffness-controlled frequency range. In contrast, attenuation of high-frequencies were dependent on the percentage of tympanic membrane in contact with the fluid. Increasing the mass in contact with the tympanic membrane resulted in decreased umbo vibration with losses between 22 and 30 dB above 0.8 kHz.

**Negative Middle Ear Pressure**

If ear canal air pressure differs from middle ear pressure (MEP) a reduction in low-frequency sound transmission occurs (Møller, 2000). If the Eustachian tube ceases to
periodically open, gas absorption within the middle ear continues; yet pressure equalization does not occur leading to a negative pressure gradient across the tympanic membrane (Magnuson, 2001). Negative MEP resulting from Eustachian tube dysfunction is one of the most common middle ear disorders. A connection between negative MEP and OME has been hypothesized (e.g., Dai et al., 2008; Magnuson, 2001).

Negative MEP is widely-distributed within the general population. This is due to both normal variations in MEP throughout the day and the high concurrence between negative MEP and the common cold (Elkhatieb, Hipoksind, Woerner & Hayden, 1993; McBride, Doyle, Hayden & Gwaltney, 1989). Furthermore, its classical association with childhood otitis media makes it the most pervasive of all types of middle ear dysfunction (Magnuson, 2001). The cumulative incidence of negative MEP between -150 and -250 mmH$_2$O has been found to be between 25 and 55%, in 7-year-olds, depending on the time of year (Lildholdt, 1980). Maximal conductive hearing loss occurs at 0.5 kHz and cannot be predicted from tympanometry (Lildholdt et al., 1979). Audiometric air-bone gaps increase systematically as MEP increases from 0 to -400 mmH$_2$O (Cooper, Langley, Meyerhoff & Gates, 1977).

Studies utilizing human cadaver temporal bones have demonstrated that the formation of pressure gradients across the tympanic membrane results in alteration of middle ear mechanics. Data has been collected at the umbo and the stapes using non-contacting optical measurement systems, video measurement systems, and laser vibrometers with negative pressure gradients ranging from -50 to -300 mmH$_2$O (Dai et al., 2008; Gan et al., 2006; Gyo & Goode, 1987; Murakami, Gyo, & Goode, 1997). Negative pressures as small as -50 mmH$_2$O reduced peak-to-
peak umbo displacement below around 1 to 1.2 kHz. As the pressure difference across the
 tympanic membrane increased, the highest frequency at which umbo displacement occurred
 shifted upward (Gan et al., 2006; Gyo & Goode, 1987; Murakami et al., 1997). With MEP equal
to -200 mmH\textsubscript{2}O the magnitude of reduction varies considerably across study from around 8 to 16
dB (Dai et al., 2008; Gan et al., 2006; Gyo & Goode, 1987; Murakami et al., 1997). Some
investigations have reported increased umbo displacement between 1.4 and 3 kHz (Gyo &
Goode, 1987; Murakami et al., 1997) or between 7 and 8 kHz (Dai et al., 2008).

Stapes displacement tended to follow a similar pattern to that of umbo displacement, but
the magnitude of changes was comparatively less (Murakami et al., 1997). LDV measurements
revealed that stapes displacement was significantly reduced under negative MEPs as small as
-100 mmH\textsubscript{2}O for frequencies less than 1.5 Hz; but at -50 mmH\textsubscript{2}O significant reductions were
found only for frequencies less than 1 kHz (Gan et al., 2006). These trends are similar to what
has been found with umbo displacement. Displacement transfer functions, a form of input-output
function referring to the ratio between umbo displacement and stapes displacement, are nearly
unaffected when MEP is shifted to negative gradients. These findings indicate that umbo and
stapes displacement are affected by the same degree when MEP is shifted negatively. Therefore,
the efficiency of sound transmission through the middle ear is independent of negative shifts in
MEP.

Conventional Clinical Tests of Middle Ear Function

The most widely employed test of middle ear function is conventional 226-Hz
tympanometry. However, acoustic reflex thresholds have also been used to confirm the presence of a conductive pathology. Although multifrequency tympanometry has shown promise in differentiating normal from abnormal ears or high impedance pathologies from low impedance pathologies, it has not gained widespread use.

226-Hz Tympanometry

Tympanometry is a technique which measures the acoustic impedance (Z) or admittance (Y) of the ear as a function of ear canal pressure for purposes of analyzing the acoustical characteristics of the middle ear system (ASHA, 1988; Haughton, 2002). Use of admittance is preferred to impedance because it allows for simpler mathematical calculations (Haughton, 2002). These two quantities are reciprocals of one another as defined in equation 1.3, below.

$$Y = \frac{1}{Z}$$ (1.3)

Acoustic admittance is defined as the ratio between volume velocity and sound pressure. Measuring the acoustic admittance using a probe placed in the external ear canal at some distance from the tympanic membrane does not present a problem when measuring at low frequencies due to the near equivalence of sound pressure throughout the ear canal. While the dekapascal (daPa) is currently the standard unit of pressure employed in clinical instrumentation, older clinical studies as well as laboratory investigations have often reported pressure in the mmH₂O unit. The relation between the two units is defined in equation 1.4 (Margolis & Hunter, 1999).

$$1 \text{ daPa} = 1.02 \text{ mmH}_2\text{O}$$ (1.4)
The clinical procedure consists of inserting a small probe into the ear canal so that a hermetic seal is obtained (Haughton, 2002; Margolis & Hunter, 1999). The probe assembly contains three tubes connected to: (a) a speaker, (b) a microphone, and (c) a pneumatic system to manipulate ear canal pressure. Conventionally, a low-frequency probe tone (e.g., 226-Hz) is presented continuously at a fixed level (e.g., 85 dB SPL) while ear canal air pressure is manipulated to create positive and negative pressure gradients across the tympanic membrane. Ear canal sound pressure is transduced into an electrical signal by the microphone within the probe assembly and then amplified (Margolis & Hunter, 1999). Deviations from the set probe tone level are compensated for by an automatic gain control. The amplified signal is then compared to the probe signal in order to determine magnitude and phase angle changes with voltage differences between the two signals being proportional to the admittance of the ear.

During the early years of tympanometry, impedance changes were measured in arbitrary units. At present, acoustic admittance is reported in the mmho or in the cm³ (or ml). The 226-Hz probe tone frequency was chosen to facilitate ease of calibration; at this frequency a hard-walled cavity with a volume of 1.0 cm³ has an acoustic admittance of 1.0 mmho. When using a low-frequency probe tone, as is common in clinical practice, changes in the stiffness of the middle ear system may be detected by changes in the morphology of the tympanogram. The most popular method for interpretation of tympanograms remains the qualitative typological system created by Jerger (1970). Within this system, the height of the tympanogram and the pressure at which peak impedance/admittance occurs are considered the most important characteristic features.

Alternatively, better test performance may be achieved by utilizing quantitative
tympanometric measurements which have come about as a result of technological advances in instrumentation (Shanks & Shohet, 2009). Quantitative analysis of tympanograms is comprised of four measurements: (a) tympanogram peak pressure, (b) equivalent ear canal volume, (c) peak compensated static acoustic admittance, and (d) tympanogram width (Margolis & Hunter, 1999; Shanks & Shohet, 2009).

_Tympanogram Peak Pressure_

The ear canal pressure at which maximum admittance is obtained is referred to as tympanogram peak pressure (TPP). Pressure applied to the tympanic membrane, irrespective of polarity, increases its stiffness, thus inhibiting the flow of energy. When the tympanic membrane is under minimal tension, admittance into the middle ear system is at its maximum (Shanks & Shohet, 2009). In normal middle ears, this occurs when there is no pressure gradient across the tympanic membrane. That is, MEP is equal to atmospheric pressure or the pressure gradient is near 0 daPa. The ear canal pressure at which maximum admittance occurs is taken as TPP which is an indirect measurement of MEP.

Discrepancies between TPP and MEP are more prevalent in ears with small middle ear volumes or highly compliant middle ear systems (Gaihede, 1999a; Gaihede, 1999b). These errors have been specifically identified in ears with otitis media (Gaihede, Bramstoft, Thomsen & Fogh, 2005; Gaihede et al., 2000). However, to date there has not been a commercially-produced technology that has been shown to identify MEPs with greater accuracy using a non-invasive methodology.


**Equivalent Ear Canal Volume**

During tympanometry the probe is placed within the ear canal, some distance from the tympanic membrane, prior to measurement. Although this is a practical location for the probe, it results in greater difficulty in the interpretation of measurements. It is the separate acoustic admittances of the ear canal and middle ear which are of primary interest; but the result of using this location is the measurement of their combined acoustic admittance (Shanks & Shohet, 2009). By applying an ear canal pressure great enough to stiffen the tympanic membrane so that it reflects nearly all of the probe tone signal, any residual admittance can be attributed to the air contained within the ear canal. When subtracted from the complex admittance quantity, the middle ear admittance can be derived.

In clinical practice, ear canal admittance at two ear canal pressures are used in the calculation of equivalent ear canal volume: TPP and +200 daPa. Error arises using this approach because a +200 daPa ear canal pressure often does not drive the middle ear impedance to infinity and thus cannot fully eliminate the admittance contributions of the middle ear (Shanks & Lilly, 1981). Equivalent ear canal volume has been reported to overestimate the true ear canal volume by as much as 39%. Shanks and Lilly (1981) have reported that error can be reduced to around 10% if the probe tone is increased from 226- to 660-Hz and if admittance at -400 daPa is used rather than admittance at +200 daPa. Nevertheless, this seemingly simple change in conventional procedure has not been widely adopted.

Clinically, it is well-known that an abnormally large equivalent ear canal volume is
consistent with perforation of the tympanic membrane or presence of a patent tympanostomy tube (Roup, Wiley, Safady & Stoppenbach, 1998; Shanks, 1984). For example, Shanks et al., (1992) obtained data from a group of 334 children younger than 7-years-old. They found that either an equivalent ear canal volume greater than 1.0 cm$^3$ or an equivalent ear canal volume increase of more than 0.4 cm$^3$ following tympanostomy tube placement was satisfactory in determining tympanostomy tube patency.

*Peak Compensated Static Acoustic Admittance*

Admittance is typically measured across a range of ear canal pressures yet it is admittance at only two ear canal pressures that are important for calculation of peak compensated static acoustic admittance: (a) TPP and (b) a high (+200 daPa) or low (-400 daPa) ear canal pressure. In clinical practice, +200 daPa is often used. The difference in admittance between measurements at +200 daPa and TPP reflects the peak compensated static acoustic admittance (Shanks & Shohet, 2009).

Acoustic admittance is inversely proportional to the amount of fluid within the middle ear space (Margolis & Hunter, 1999). When a large amount of fluid has accumulated within the middle ear, tympanometry will often display a flat trace where acoustic admittance is invariant to changes in ear canal pressure (Margolis & Hunter, 1999; Møller, 2000). Conventional 226-Hz tympanometry may be less sensitive to OME when the middle ear is only partially-filled with fluid. For example, tympanometric measurements in human temporal bones revealed that there must be at least 0.5 ml of fluid (about five-sixths of the temporal bone middle ear volume) within
the middle ear before static admittance is reduced below normal values (Dai et al., 2007). Clinical investigation in children with histories of chronic or recurrent otitis media revealed that using a cutoff criterion of less than or equal to 0.3 mmho resulted in a sensitivity of 70% and a specificity of 80% (Nozza, Bluestone, Kardatzke & Bachman, 1994).

**Tympanogram Width**

The fourth quantitative variable derived from tympanometry is tympanogram width. Although there are several ways to measure tympanogram width, a simple and popular method involves determining the pressure interval between the rising and falling tympanogram slopes at one-half of the peak compensated static acoustic admittance (Shanks & Shohet, 2009). Of all the tympanometric measurements, it is tympanogram width that has demonstrated the greatest accuracy in detecting the presence of OME.

Nozza et al. (1994) reported sensitivities and specificities in 249 ears with history of chronic or recurrent OME. Using myringotomy as the gold standard for diagnosis of OME and setting a tympanogram width criterion where widths greater than 275 daPa are abnormal, an 81% sensitivity and 82% specificity were achieved. This study demonstrated that tympanogram width was more accurate than peak compensated static acoustic admittance; but due to the high incidence of middle ear disease in childhood, superior test performance should be actively sought.
The acoustic reflex refers to the contraction of the stapedius muscle in response to a high intensity auditory stimulus (Roush & Grose, 2006). Contraction of the middle ear muscles results in a stiffening of the ossicular chain which decreases the admittance measured at the probe. Clinical measurements have often used a 226-Hz probe tone, although other probe-tone frequencies may be used. The stimulus level at which changes in admittance can be detected reliably around 50 to 75% of the time defines the acoustic reflex threshold. Commonly used activator stimuli include pure-tones and broadband noise which may be delivered either ipsilaterally or contralaterally.

Presence of middle ear dysfunction often precludes the detection of the acoustic reflex due to attenuation of the activating stimulus and/or because increased stiffness resulting from middle ear pathology precludes the detection of small changes in admittance. Jerger, Anthony, Jerger, and Mauldin (1974) reported the percentage of ears in which acoustic reflex thresholds could be determined for individuals with unilateral conductive hearing loss. The normal ear was presented the reflex-activating stimulus while the reflex was measured in the ear with conductive impairment. While reflexes could be detected in 25% of ears with a 10 dB air-bone gap, reflexes could be detected in only 5% of ears with a 35 dB air-bone gap. Consequently, middle ear pathologies which produce conductive hearing loss often result in absent acoustic reflexes. However, absence of reflexes may also be due to cochlear or retrocochlear dysfunction thus reducing, but not excluding, its clinical utility.
Multifrequency Tympanometry

When tympanometry is performed at multiple probe-tone frequencies with individual admittance components analyzed rather than the complex admittance, tympanograms may be analyzed using the Vanhuyse model and the resonance frequency of the middle ear system may be determined (Margolis & Hunter, 1999). Admittance components are susceptance (B) and conductance (G) and are related to admittance as defined in equation 1.5.

\[ Y = \sqrt{G^2 + B^2} \]  

(1.5)

Although B and G may be thought of as the admittance counterparts of \( X_s \) and \( X_m \), they do not form a reciprocal relationship (Rosowski & Relkin, 2001). Rather the relationship may be defined as follows:

\[ G = \frac{R}{R^2 + X^2} \] and \[ B = \frac{-X}{R^2 + X^2} \]  

(1.6)

Research by Vanhuyse, Creten, and Van Camp (1975) has demonstrated a series of characteristic notching patterns that occur in the normal ear as probe tone frequency increases. Subsequent investigations have shown that the optimal method for determining the middle ear resonance frequency is to determine the probe-tone frequency which yields a notch in B equal to the positive tail of the tympanogram (Margolis & Hunter, 1999). Resonance frequencies determined using these methods have shown that simple high impedance middle ear pathologies (e.g., ossicular fixation) have abnormally high resonance frequencies whereas simple low impedance middle ear pathologies (e.g., ossicular disarticulation) have abnormally low resonance frequencies but for both types of dysfunction there is a considerable degree of overlap with normative data. However, these simplified explanations do not hold for more complex types of
middle ear dysfunction (e.g., OME) in which both mass and stiffness characteristics are altered.

Although utilization of multifrequency tympanometry by clinical audiologists has been recommended and said to be cost-effective (Hanks & Robinette, 1993), widespread adoption has not occurred. It is possible that this is due to lack of familiarity with the procedure and/or less straightforward interpretation of results compared to conventional 226-Hz tympanometry. In response, some commercial manufacturers have included an algorithm in select models of their middle ear analyzers to automatically determine middle ear resonance frequency. Using one of these automatic algorithms, Ogut et al. (2008) measured middle ear resonance frequencies from 100 normal ears and 25 surgically-confirmed otosclerotic ears. Using a cutoff frequency of 1.025 kHz, they found a sensitivity of 80% and a specificity of 82%. This performance is superior to conventional tympanometry, but not sufficient to use as the sole indicator on which to base candidacy for surgical intervention.

Limitations of Conventional Clinical Middle Ear Tests

Single-frequency 226-Hz tympanometry has been a popular tool for evaluation of middle ear function for many years. Its diagnostic value has been investigated in a variety of middle ear pathologies over several decades (for review, see Margolis & Hunter, 1999). Its greatest strength has been its ability to detect OME but its sensitivity has often been reported to be inferior to that of pneumatic otoscopy performed by an experienced otoscopist. Since not all clinicians who diagnose OME are equally skilled at otoscopic examination (Steinbach et al., 2002), an objective measure with a high degree of accuracy is needed.
Further, tympanometry is not well suited for detection of ossicular chain pathologies. For example, use of 226-Hz tympanometry in otosclerotic ears shows poor diagnostic accuracy (Margolis & Hunter, 1999). When stapes fixation occurs resulting in corresponding increases in impedance, the overall effect on input impedance at the tympanic membrane is minor. Peak compensated static acoustic admittance has been investigated to assess its ability to distinguish otosclerotic ears from normal ears. Muchnik, Hildesheimer, Rubinstein, and Gleitman (1989) reported that only 14 out of 42 otosclerotic ears were identified with abnormally low static admittance. Browning, Swan, and Gatehouse (1985) compared 34 individuals with surgically-confirmed otosclerosis and 34 age- and sex-matched controls. They found that only 38% of otosclerotic ears fell below 0.2 ml which yielded a 90% specificity. Shahnaz and Polka (2002) reported outcomes from a sample of 68 healthy ears and 36 otosclerotic ears. Sensitivity and specificity were 74% and 54%, respectively. These findings suggest that peak compensated static acoustic admittance used in isolation may be a poor test for distinguishing normal ears from ears with stapes fixation. Assessment of middle ear pathology could be improved by developing objective methods with superior test performance.

In conventional tympanometry, information is obtained at a single low-frequency whereas multifrequency tympanometry may provide information up to 2 kHz (Keefe & Feeney, 2009). However, for frequencies greater than 1.5 kHz, ear canal standing waves create large deviations in sound pressure over relatively short distances between the tympanometry probe and the tympanic membrane. These variations prevent valid measurements at higher frequencies due to the interaction between ear canal length and probe insertion depth (Keefe & Feeney, 2009; Margolis
Hunter, 1999). Although probe frequencies up to 2 kHz have been successfully used, there is no calibration standard for these higher probe frequencies (Keefe & Feeney, 2009). Frequencies higher than 2 kHz provide an important contribution to speech and music perception as well as environmental awareness. The capability to accurately measure these frequencies may prove invaluable in understanding the effects middle ear dysfunction on sound transfer at high-frequencies.

Wideband Energy Reflectance as a Measure of Middle Ear Function

Under normal conditions sound travels through the external auditory canal and is transmitted through the middle ear system prior to electromechanical transduction within the cochlea. The middle ear acts as an impedance transformer to increase the efficiency of sound transmission between the low impedance air of the ear canal and high impedance fluids of the cochlea (Møller, 2000). Although the middle ear possesses a high level of energy efficiency it is not a perfect system (Allen, Jeng, & Levitt, 2005). While a fraction of the acoustic energy is absorbed by the middle ear, the remainder is reflected back into the external auditory canal. The ratio of the power reflected to the total incident power is referred to as the energy reflectance (ER) ratio (Keefe & Feeney, 2009; Stinson, 1990; Voss & Allen, 1994). Measurements of ER range from 0 (or 0%) indicating that no energy is reflected to 1 (or 100%) indicating that all energy is reflected. The ER technique utilizes a broadband stimulus (e.g., click or chirp) presented at a constant sound pressure level (around 55 to 60 dB SPL), with resulting ER values plotted as a function of frequency.
An important advantage of ER measurements compared to conventional admittance/impedance measurements (i.e., tympanometry) is that measures of reflectance are not significantly affected by ear canal location. In contrast, admittance/impedance measurements are subject to the influence of ear canal standing waves thus limiting measurements at frequencies higher than 1.5 kHz (Keefe & Feeney, 2009; Stinson, Shaw, & Lawton, 1982; Voss, Horton, Woodbury & Sheffield, 2008). For example, a comparison of admittance and reflectance measurements in cats at different measurement sites revealed that admittance measurements made near the ear canal entrance were accurate below 3 kHz, but reflectance measurements maintained acceptable accuracy up to 5 kHz (Huang, Rosowski, Puria, & Peake, 2000). These findings suggest that reflectance measurements are more resistant to the effects of ear canal standing waves compared to admittance measurements.

Thus far, the ER test has been used to document age-related changes in the middle ear (Feeney & Sanford, 2004; Keefe & Levi, 1996; Keefe, Bulen, Arehart, & Burns, 1993; Sanford & Feeney, 2008), in detecting middle ear muscle reflexes at lower levels compared to conventional admittance techniques (Feeney & Keefe, 1999; Feeney & Sanford, 2005; Feeney, Keefe & Sanford, 2004), and in identifying middle ear dysfunction in both children and adults (Allen et al., 2005; Feeney, Grant, & Marryott, 2003; Hunter & Margolis, 1997; Keefe & Simmons, 2003; Margolis, Saly, & Keefe, 1999; Piskorski, Keefe, Simmons & Gorga, 1999; Shahnaz et al., 2009).

**Wideband Energy Reflectance in Normal Ears**

ER varies, due to and along with impedance variations, as a function of frequency (Allen
et al., 2005). For normal, young-adult middle ears ER tends to be near 1 at the lowest frequencies. For frequencies less than 1 kHz, the stiffness reactance of the annular ligament is thought to be the major contributor to middle ear impedance (Lynch, Nedzelnitsky, & Peake, 1982). ER declines with increasing frequency to its lowest ER values between 1 and 5 kHz where impedance is dominated by resistance (Allen et al., 2005). ER increases with increasing frequency above 4 kHz with mass reactance providing a greater contribution to impedance than found at lower frequencies.

Wideband Energy Reflectance in Low Impedance Pathologies

Feeney et al. (2003) reported two case studies in which ER measurements were obtained from participants with perforated tympanic membranes. A 21-year-old woman presented with a right tympanic membrane perforation in the presence of tympanosclerosis but no active infection. The second participant presented with a left posterior-medial perforation less than 0.5 mm in diameter. Tympanometry confirmed the presence of both perforations with abnormally high estimates of equivalent ear canal volume and a lack of distinct peaks in the traces. In these ears ER was below the 95% confidence intervals for frequencies below 0.841 kHz. A minimum in the ER versus frequency function occurred around 0.3 kHz where ER was equal to 0. This pattern is similar to what Voss et al. (2000) found when comparing earphone sound pressure levels measured in the canals of ears with normal and perforated tympanic membranes. Ears with small (1-3%) perforations evidenced lower sound pressure levels for frequencies below 1 kHz and a similar notching occurred around 0.3 kHz.
Allen et al. (2005) reported ER in a female subject with a 3 to 4 mm tympanic membrane perforation in an anterior-inferior-central position. ER ratios from her right ear with the perforation were reduced for frequencies below 1.5 kHz compared to measurements from both her left ear and a standard coupler. Although ER ratios in her perforated ear did not closely resemble those reported by Feeney and colleagues (2003), normalized impedance did demonstrate a distinct notch around 0.35 kHz. This is similar to what was found in reports by both Feeney et al. (2003) and Voss et al. (2000).

When ossicular disruptions are present in the middle ear system, conventional low-frequency tympanometry may reveal abnormally large peak compensated static acoustic admittance and absent ipsilateral middle ear muscle reflexes. However, low-frequency tympanometry is not accurate at detecting ossicular disruptions. Previous reports have pointed out its poor performance at distinguishing normal middle ears from those with ossicular disruptions (Lidén, Harford, & Hallén, 1974; van de Heyning, van Camp, Creten, & Vanpeperstraete, 1982). Middle ear muscle reflexes are sensitive to various types of middle ear dysfunction but lack specificity. Their absence may be due to sensorineural, retrocochlear, or middle ear dysfunction. Contrastingly, ER measurements have shown promise in their ability to distinguish ears with ossicular discontinuities from normal ears.

Feeney, Grant, and Mills (2009) measured ER in 5 human cadaver temporal bones from adults greater than 60-years of age. Measurements were made initially as a baseline, again after interruption of the ossicular chain with an argon laser, and finally after reconstruction of the ossicular chain using glass ionomeric cement. Following the creation of 2 mm incudostapedial
separations in the ossicular chains, ER measurements averaged from all five temporal bones displayed a 31% reduction at 0.63 kHz where an ER notch emerged. A peak also occurred at 1.414 kHz where a 9% increase in ER appeared. ER was nearly restored (i.e., around 10% less than initial baseline) following repair of the incudostapedial joints.

A case study reported earlier by Feeney and colleagues (2003) corroborated these findings in human temporal bones. A 26-year-old woman with history of bilateral otosclerosis was evaluated due to interruption of the left ossicular chain following a slipped prosthesis. Pure-tone audiometry revealed a moderately-severe conductive hearing loss, whereas tympanometry was within normal limits. ER ratios in the left ear were abnormally low between 0.375 and 0.794 kHz with a notch centered at 0.667 kHz. ER was also higher than normal between 3.775 kHz and 4.757 kHz. Comparison with one of the temporal bones from Feeney et al. (2009) showed a striking similarity in ER versus frequency functions. The emergence of low-frequency notches around 0.65 kHz are consistent with lowering of the middle ear resonance frequency due to decreased middle ear stiffness. This lowered middle ear resonance is consistent with previous investigations utilizing multifrequency tympanometry in cases of ossicular disarticulation (Funasaka & Kumakawa, 1988).

Energy Reflectance in High Impedance Pathologies

Feeney et al. (2003) reported ER as a function of frequency in two cases of otosclerosis. Audiometric results from a 49-year-old woman with right-ear otosclerosis were consistent with a moderate mixed hearing loss and normal 226-Hz tympanometry. ER ratios were higher than the
95th percentile of normative data from 0.25 to 0.944 kHz. Findings from a 26-year-old woman with right-ear otosclerosis were in agreement with a mild, mixed hearing loss and abnormally low peak compensated static acoustic admittance per 226-Hz tympanometry. ER ratios were also higher than the 95th percentile of normative data across a similar bandwidth, 0.25 to 1.189 kHz. ER was higher than normal at 4.757 kHz and from 5.993 to 7.551 kHz. Allen et al. (2005) reported on a woman in her 20s with bilateral otosclerosis. ER ratios in both ears was higher than normal between 0.4 and 1.5 kHz.

Shahnaz et al. (2009) determined test performances for the ER test, multifrequency tympanometry, and 226-Hz tympanometry for differentiating normal and otosclerotic ears. Data were collected from 62 normal hearing ears and 28 otosclerotic ears. ER ratios were presented for one-third octave bands (0.211 to 6 kHz). Multifrequency tympanometry variables were middle ear resonance frequency and the frequency at which resistance was equal to reactance (i.e., F45°). Conventional 226-Hz tympanometry measures included peak compensated static acoustic admittance and tympanogram width. Both multifrequency tympanometry and ER produced superior test performance than 226-Hz tympanometry. ER was significantly larger below 1 kHz for otosclerotic ears. The ER test achieved a sensitivity and specificity of 82% and 83%, respectively. Combining multifrequency tympanometry and ER resulted in perfect accuracy for differentiating otosclerotic from normal ears.

Shahnaz, Longridge, and Bell (2009) also compared ER versus frequency functions in 15 otosclerotic patients before and after stapes surgery. Following corrective surgery, ER ratios were reduced between 0.7 and 1 kHz with a small increase between 2 and 4 kHz. The ER reduction
between 0.7 and 1 kHz produced an overlap between the postoperative and normal groups consistent with otosclerotic ears more closely approximating a system with normal middle ear sound transmission following corrective surgery.

Several authors have reported ER results in individual cases of OME; but few reports have emerged detailing group data. Feeney et al. (2003) reported test results from four ears of three adults with OME, but with varied histories and audiometric findings. ER measurements at ambient pressure were similar across the three participants with reflectance elevated to near 1.0 for frequencies less than 4 kHz. A narrowband notching above 4 kHz created some overlap with normative data. Pediatric findings from children between 2.5- and 5-years have been reported with ER higher than normal across a wide frequency range, but with a similar characteristic notch around 4 kHz (Allen et al., 2005; Jeng, Levitt, Lee, & Gravel, 2002). These reports are consistent with the higher than normal ER ratios between 1 and 4 kHz recorded in infants and young children with both cleft palate and OME (Hunter, Bagger-Sjoback, & Lundberg, 2008).

Negative MEP may be considered a high impedance pathology under some circumstances. However, variation of MEP throughout the day is also a normal phenomenon. Negative MEP is quantified via tympanometry. Tympanogram peak pressure has been used to determine the degree of pressure deviation from ambient pressure. Although discrepancies between TPP and MEP have been reported (Decraemer, Creten & Van Camp, 1984; Gaihede, 2000; Kobayashi, Okitsu & Takasaka, 1987), it remains the most commonly used non-invasive measurement technique for ascertaining MEP (Martin, Champlin & Chambers, 1998). Although TPP is a reasonably accurate indicator of MEP, it provides little useful information on the effects
of altered MEP on sound transmission through the middle ear system. In contrast, ER measurements provide frequency-specific information on the acoustic energy that is transmitted into the middle ear system.

Feeney et al. (2003) reported the case of a 52-year-old woman with a bilateral sensorineural hearing loss in the high-frequencies. She presented with bilateral negative MEP: -155 daPa in the right ear and -105 daPa in the left ear. ER ratios were higher than normal from 0.334 to 0.891 kHz for the right ear and from 0.25 to 0.297 kHz for the left ear. ER ratios were also lower than normal from 2.119 to 3.775 kHz for the left ear. Beers and colleagues (2010) performed tympanometry and ER in school-aged children. They grouped ears by MEP with -100 to -199 daPa classified as mild and ≤ -200 daPa categorized as severe negative MEP. Mean ER for mild (n=30 ears) and severe (n=24 ears) negative MEP were significantly higher than mean ER in normal ears (n =144 ears) for frequencies from 0.63 to 2 kHz and 0.63 to 5 kHz, respectively.

Statement of the Problem

Due to the high prevalence and incidence of negative MEP within the general population, it is important to gain a thorough understanding of its effects on sound transmission so that the test performance of new diagnostic techniques may be maximized. Further, the ability to use a simple compensation procedure to overcome the confounding effects of negative MEP may help to enhance diagnostic accuracy within clinical populations. Information comparing clinical and normative samples is not sufficient due to the high co-occurrence of negative MEP and OME. It
would be ideal to determine the effects of negative MEP and the validity of a compensation procedure on a normal adult sample.

Effects of Negative Middle Ear Pressure

Negative MEP alters the mechanics of the middle ear system resulting in alterations in sound conduction to the inner-ear (Gan et al., 2006; Gyo & Goode, 1987; Murakami et al., 1997). However, much of this information is derived from studies in human temporal bones. Due to ethical concerns, few studies have been able to elucidate the effects of negative MEP on sound transmission in live human subjects. One such study utilized distortion product otoacoustic emission measurements, which reflect the combined effects of forward and reverse sound transmission, to investigate alterations in sound transmission resulting from experimentally-induced negative MEP gradients in normal subjects (Sun & Shaver, 2009). Distortion product otoacoustic emissions were measured when MEPs were near ambient pressure and when negative pressure shifts were induced by having subjects perform either a Toynbee or a closed-nostril sniffing maneuver. Various degrees of negative MEP were induced with largest reductions occurring at 1 kHz and below with minimal changes at 2 kHz. Some ears demonstrated an enhancement at 8 kHz which is consistent with some LDV measurements of the umbo made in human cadaver temporal bones (Dai et al., 2008).

ER measurements do not reflect the complete forward transmission pathway, but do have several advantages over otoacoustic emission measurements: (a) the measurement procedure is substantially shorter in duration, (b) signal-to-noise ratios are high even at low frequencies and
when middle ear impedance is altered, and (c) there is no confounding effect of the reverse acoustic transfer function. To date, there has not been a systematic study investigating the effects of negative MEP on ER measurements in normal subjects. However, the results of simulated negative MEP using ER have been reported (Margolis et al., 1999; Margolis et al., 2001; Sanford & Feeney, 2008). For example, Margolis and colleagues (1999) pressurized ear canals with +300 daPa and found that ER decreased between 3 and 8 kHz, but increased at lower frequencies. However, it is unclear whether these data are representative of actual negative MEPs. Although a positive ear canal pressure should create a similar tympanic membrane position relative to a negative MEP, the actual MEP would be positive (re: ambient pressure) resulting in a medial displacement of both the oval and round window membranes. For many years it has been assumed that these conditions are comparable in terms of their effects on sound transmission; to date, no published study has verified this assumption.

Compensation of Negative Middle Ear Pressure

In order to achieve satisfactory test performance for separating normal and pathological middle ears, it is imperative that middle ear dysfunctions with a high incidence and/or prevalence, but a minor and transient impact on auditory function, be well-understood and compensated for whenever possible. This should prevent deterioration of test performance during the assessment of middle ear pathologies which may require medical and/or surgical intervention. An important consideration when using acoustic measurements to assess middle ear status is whether or not modifications in the measurement need to be made to compensate for the
presence of negative MEP. Since MEP variation throughout the day is considered a normal occurrence (Sakikawa, Kobayashi & Nomura, 1995), a compensation technique should reduce the measurement variability within a normal sample.

Applying ear canal air pressure to counteract non-zero MEPs is standard procedure when measuring middle ear muscle reflex thresholds using impedance/admittance procedures. Doing so allows for the detection of reflexes at lower levels compared to measurements made at ambient pressure (Martin & Coombes, 1974; Rizzo & Greenberg, 1979; Ruth, Tucci, & Nilo, 1982). A similar compensation procedure for use with evoked otoacoustic emission measurements has been shown to improve transient evoked otoacoustic emission detectability in children with negative MEP (Hof, Anteunis, Chenault & van Dijk, 2005) and to restore distortion product otoacoustic emission amplitudes near baseline values in a non-clinical sample of college students (Sun & Shaver, 2009).

In particular, it is widely accepted that the accumulation of middle ear effusion is a common sequela of otitis media. However, the concurrence of otitis media with negative MEP is both controversial and consistent with the classical *hydrops ex vacuo* theory which postulates that the air within the middle ear continues to be absorbed, perpetually (Magnuson, 2001; Takahashi et al., 1991). If the Eustachian tube does not open then MEP becomes increasingly negative until transundation results in accumulation of middle ear effusion.

Both human temporal bones measurements in the presence of middle ear fluid and resultant modeling work suggest that a negative MEP is necessary to produce the clinically-observed low-frequency hearing losses associated with otitis media (Ravicz et al., 2004). The
combined effects of middle ear fluid and altered MEP on middle ear mechanics have been described using LDV to measure umbo velocity in human temporal bones (Dai et al., 2008). The combination of a middle ear half-full (0.3 ml) of fluid simulating serous effusion as well as experimentally-induced negative MEP resulted in a reduction in umbo displacement across all frequencies compared to control measurements. The combination of middle ear fluid and an MEP equal to -200 mmHg resulted in a 6-to 11-dB reduction in umbo displacement between 0.2 and 0.5 kHz when compared to measurements made in either the control group or the middle ear fluid only group.

Use of ear canal air pressure compensation to address negative MEP in children with probable OME has been infrequently reported. ER measurements were performed on a 9-year-old girl and a 10-year-old boy, both with recurrent OME and negative MEP. ER versus frequency functions were similar to normative ER measurements under positive ear canal pressure (Hunter & Margolis, 1997; Margolis et al., 1999). Only after ear canal pressure compensation could ER measurements be compared to normative data and determined to be abnormal due to middle ear pathology rather than simple negative MEP. To date, no systematic investigations have been performed to determine if this compensation procedure can restore ER to baseline values across a wide frequency range.

**Objectives**

This study has three goals: (a) to determine the immediate test-rest reliability of the ER measurement, (b) to clarify the frequency-dependent effects of negative MEP on ER
measurements, and (c) to determine if the ear canal pressure compensation procedure in ears with negative MEP restores ER measurements to their baseline values. To answer these questions, ER measurements were obtained from a group of young, normal ears when MEP was normal and when negative MEP was experimentally self-induced. Measurements were made at both ambient pressure and while ear canal pressure was swept from higher than to lower than ambient pressure.
CHAPTER 3

METHODS

Participants

Fifty-four adults between the ages of 18- and 35-years-old were recruited for this study. Participants were recruited through responses to fliers posted on the university campus as well as through word-of-mouth. Participants were required to (a) be in good general health, (b) have no history of chronic middle ear disease, and (c) have no known history of hearing loss. This information was obtained through participant self-report.

Screening and Experimental Procedures

Prior to testing all participants were given a written explanation of test procedures as well as potential risks and benefits. Any questions were answered by the tester. A copy of the informed consent document, approved by the university's institutional review board, was signed by the participant prior to data collection. Participants were provided $10.00 per 1 hour session for their participation. Testing was conducted in a double-walled, sound-treated booth within the Speech Science Laboratory on the first floor of Hubbard Hall located on Wichita State University's main campus. Participant report on the case history form was used as evidence of good general health. Indications of audiologically-relevant disease were used as grounds for participant exclusion. Prior to hearing screening, participants' ears were visually inspected for occlusion or any gross abnormality of the ear using a Heine mini 2000 otoscope. In instances
where tympanic membrane scarring or excessive cerumen was present in only one ear, the contralateral ear was still considered for testing.

_Hearing and Middle Ear Screening_

Pure-tone audiometry using an Interacoustics AC40 audiometer connected to EAR-3A insert earphones was used to determine air-conduction hearing thresholds at octave intervals from 0.25 to 8 kHz. A standard 5-up, 10-down threshold seeking procedure was used. Thresholds were required to be less than 25 dB HL at all test frequencies for participant inclusion.

Middle ear screening was assessed using a GSI-33 Middle-Ear Analyzer (version 1). Pressure was swept from +200 daPa to -400 daPa at a rate of 50 daPa/s. Tympanometric inclusion criteria required that peak compensated static acoustic compliance fall between 0.3 and 1.7 cm$^3$ and equivalent ear canal volume was less than 2.1 cm$^3$ (Margolis & Hunter, 1999). TPP was required to be within +/- 25 daPa of ambient pressure which is a more conservative requirement compared to clinical normative data (Sun & Shaver, 2009). Data were obtained from either one or both ears, depending on time constraints. If one ear did not meet inclusion criteria data were obtained from only one ear. Specifically, participant inclusion criteria included (a) no indications of audiologically-relevant disease per case history, (b) no contraindications per otoscopic inspection (e.g., excessive wax or obvious tympanic membrane scarring), (c) normal audiometric results, and (d) normal tympanometric results.
**Experimental Instrumentation**

All energy reflectance (ER) measurements were obtained from subjects meeting the inclusion criteria described above. The ER measurement system is a research prototype model developed by Interacoustics and Douglas Keefe of Boys Town National Research Hospital/Sonicom, Inc. An extensive description of the system has been reported elsewhere (Liu et al., 2008), but an overview is provided here. A Fujitsu Siemens workstation with a 2.53 GHz Intel Core 2 Duo processor houses a CardDeluxe (Digital Audio Labs) sound card with 24-bit A/D and D/A converters operating at a sampling rate of 22.05 kHz. The Reflwin (build number 2.38) application facilitates data collection and runs on the Windows XP operating system. Analog input and outputs from the sound card are connected to an Interacoustics AT235 middle ear analyzer with its firmware modified by the manufacturer to allow for coordination with the workstation. A custom ER probe was connected to the AT235 and was used for ER measurements. Within the ER probe assembly there is a speaker for presenting acoustic stimuli and a microphone for recording acoustic responses. Hollow tubing within the cable allows for manipulation of ear canal air pressure by providing a link between the pressure pump within the tympanometer and the termination at the probe tip. Rubber probe tips of various diameters were used to couple the probe assembly to participants' ear canals.

Calibration was performed daily using a two-tube procedure prior to data collection, as described in detail by Keefe and Simmons (2003). An ER probe tip was fitted to the probe and inserted into calibration tubes with a diameter of 7.9 cm, which is similar to the diameter of the typical adult ear canal. The probe was first inserted into the long calibration tube (~295 cm).
where 64 trials were recorded in response to click stimuli presented at a rapid rate. The probe was then removed and inserted into the short calibration tube (~8.4 cm) where another 64 trials were recorded. Measurements recorded from the longer tube represent a condition where reflections do not significantly contribute to the recorded response, whereas measurements from the shorter tube are heavily influenced by the multiple reflections between the closed end of the tube and the probe assembly. Performing measurements within these two tubes with known acoustic loads allows for the estimation of the characteristic impedance of the ear canal ($Z_c$).

$$Z_c = \rho c / S$$  \hspace{1cm} (2.1)

In equation 2.1, $\rho$ is the density of air, $c$ is the velocity of sound, and $S$ is an acoustic estimate of the cross-sectional area of the ear canal based on probe tip size (Keefe & Feeney, 2009). When the complex acoustic impedance ($Z_a$) is obtained from ear canal measurements, the acoustic pressure reflectance ($r_a$) can also be defined by equation 2.2.

$$r_a = (Z_a - Z_c) / (Z_a + Z_c)$$  \hspace{1cm} (2.2)

Acoustic energy reflectance (ER) can then be established by squaring the absolute acoustic pressure reflectance ($r_a$).

$$EA = r_a^2$$  \hspace{1cm} (2.3)

ER stimuli consisted of wideband clicks presented at a rate of approximately one every 46 ms with a total of 32 clicks presented for each run (Liu et al., 2008). The stimulus spectrum within the ear canal was designed to be fairly flat between 0.226 and 8 kHz. Responses were averaged to increase the signal-to-noise ratio. Artifacts, such as those due to respiration, swallowing, and movement, were rejected based on a median-absolute-deviation test (Liu et al.,
2008). The tester was notified by the Reflwin software to remove and reinsert the probe if it detected a leaky probe fit under ambient measurements. ER measurements were made at both ambient pressure (aER) and while pressure was swept from +200 to -300 daPa at a rate of 75 daPa/s (referred to as dynamic ER (dER)). The test ear was selected by the experimenter based on which ear had better hearing thresholds and/or less cerumen. When thresholds between ears were equivalent and cerumen buildup was not present in either ear, the test ear was selected randomly.

Experimental Protocol

For each test session, a minimum of five aER measurements and two dER measurements were made. One dER and three aER measurements were made prior to MEP manipulation, one dER and one aER measurement were made while MEP was altered, and one aER measurement was made after MEP had returned to near its initial baseline value. The following sequence provides a more detailed description of the experimental protocol.

1. dER with normal MEP (dynamic pressure baseline)
2. aER with normal MEP (first ambient baseline)
3. Probe removed and reinserted - aER with normal MEP (second ambient baseline)
4. aER with normal MEP (third ambient baseline;)
5. Negative MEP was self-induced by participants via execution of a Toynbee maneuver (swallowing with nostrils held shut) or forceful sniffing followed by swallowing with the nostrils held shut (Sun & Shaver, 2009). Concurrently, the
ear canal were pressurized to +200 daPa to facilitate the induction of a negative MEP. Through application of ear canal pressure, the tympanic membrane should have been displaced medially resulting in a positive MEP. If the Eustachian tube opens during swallowing or sniffing, at least a partial release of this positive MEP should have occurred so that when the Eustachian tube closed and positive ear canal pressure was removed, the MEP was below ambient pressure. When a negative MEP was successfully achieved, step 6 commenced. Otherwise, step 5 and all subsequent steps were repeated for up to 15 minutes at which point if the participant remained unsuccessful at achieving and maintaining a negative MEP, the protocol was discontinued.

6. dER with negative MEP (experimental condition)

7. aER with negative MEP (experimental condition)

8. MEP equilibrated by swallowing five or more times.

9. aER with normal MEP (fourth ambient baseline)

During each aER, dER, or tympanometric measurement participants were asked to refrain from talking, swallowing, or making any orofacial movements. The MEP was verified indirectly by use of TPP. Tympanometry was measured immediately before steps 1, 5, 6, 8, and 9 to establish TPP. Some participants were asked to return for an additional test session when the tester suspected that the participant may be able to easily self-induce negative MEPs, create different magnitudes of negative MEP, and maintain negative MEP for several minutes during subsequent testing. For those participants who were unable to self-induce and maintain a
negative MEP in step 5, only baseline data were collected and analyzed. Data was discarded
when the difference in TPP between steps 1 & 5 or steps 6 & 8 was greater than 25 daPa.

Data Processing

Fast Fourier transformation was automatically performed by the ReflWin software with
an analysis bandwidth ranging from 0.223 to 8 kHz. A frequency resolution of 12 points per
octave was manually selected. Each ER data file was exported from the ReflWin software into
.xls files. Data were processed and statistical analyses were performed using the Gnumeric 1.10.1
spreadsheet application. TPP data were manually entered into the spreadsheet. Subsets of data
were transferred to gnuplot 4.2 and QtiPlot 0.9.7.10 for creation of various graphical plots.

Statistical Analyses

Inferential statistical analyses were performed only at half-octave frequencies for
purposes of data reduction. Strictly speaking, these frequencies were not exactly at half-octaves.
Frequencies chosen for analyses were those closest to half-octave frequencies: 0.2649, 0.3746,
0.5, 0.7492, 1, 1.498, 2, 2.997, 4, 5.993, and 8 kHz. For simplicity of reporting and comparison to
other published reports, these frequencies were rounded to the nearest half-octave and reported
as 0.25, 0.375, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 kHz. Furthermore, it should be noted that data
were not averaged across half-octave bands; they were averaged across twelfth-octave bands with
data at half-octave frequencies selected for statistical analyses.

The Shapiro-Wilk test was used to screen a portion of the total dataset for deviations from
the expected normal distribution. Pearson correlation coefficients were used to measure the
association between test and retest measurements (step 2 vs. step 3, step 3 vs step 4). Paired t-
tests were used to compare ER at half-octave frequencies between ears with normal MEP, ears
with negative MEP, and ears with compensated negative MEP (step 3 vs 9, step 3 vs 6, step 3 vs
7, step 6 vs 7). Paired t-tests were also used to compare aER measurements with dER
measurements (step 1 vs step 2, step 6 vs 7). An alpha level of 0.05 was adopted for Pearson
correlation and 0.01 for Shapiro-Wilk and paired t-tests.
Normative Study of Energy Reflectance Measurements

Ambient pressure energy reflectance (aER) measurements were made on all subjects meeting the inclusion criteria. Of the 53 volunteers, 48 met the inclusion criteria with the remaining 5 excluded due to hearing loss, history of otitis media, or difficulty obtaining an adequate hermetic seal during dynamic energy reflectance (dER) measurements. One ear from each of the 48 subjects was used for all subsequent measurements.

Screening data for normality was performed on a subset of the total dataset prior to statistical analyses. Mean data from steps 3 and 7 were subjected to both objective and subjective testing to determine if the data were drawn from a normally-distributed population. Half-octave frequencies were selected from these datasets which included 48 ears with normal MEP (step 3) and 17 ears with negative MEP between -40 and -65 daPa (step 7).

For ears with normal MEP, the Shapiro-Wilk test indicated significant deviations from normality at 3 out of 11 frequencies tested: 0.25, 6, and 8 kHz ($p < 0.01$). To further examine normality of the data, graphical analyses were performed by generating Q-Q plots for each frequency. For the normal MEP group, deviations from the hypothetical normal distribution were most apparent for a single-tail at 8 kHz. At 6 kHz, deviations were smaller in magnitude but present at both tails. At 0.25 kHz, deviations were qualitatively similar to those found at 8 kHz but smaller in magnitude. For the negative MEP group the same set of frequencies (0.25, 6, and 8
kHz) were found to have non-normal distributions at the $p = 0.01$ level. Q-Q plots in ears with negative MEP were similar to those in ears with normal MEP. Data points in ears with negative MEP did not cluster around the 45° slope as tightly as ears with normal MEP, possibly owing to the smaller sample size in the latter group. Since only 3 out of 11 frequencies in each screened dataset were considered non-normal, parametric statistics were utilized.

Mean energy reflectance (ER) ratio at ambient pressure as a function of frequency (taken from step 3) for 48 ears is displayed in Figure 1. The configuration of the mean aER versus frequency function is characterized by high ER ratios at low-frequencies, all above 0.8 for frequencies below 0.45 kHz. Above 0.4 kHz ER ratio declines monotonically with increasing frequency until it plateaus at around 1.1 kHz where ER drops to approximately 0.35 and fluctuates only minimally through 2.1 kHz. As frequency further increases, ER declines to a minimum of 0.24 at 2.997 kHz. As frequency increases above 2.1 kHz, ER ratio increases monotonically to 0.83 at 5.993 kHz. At higher frequencies the ER versus frequency function shifts again whereby ER begins to decrease with increasing frequency.

Mean dER taken from 46 ears in step 1, ER measured as a function of both frequency and ear canal pressure, is shown in a contour plot in Figure 2. Visual inspection of this plot suggests a general trend: as ear canal pressure increases or decreases, the configuration of the ER versus frequency function changes little but shifts upward in frequency. ER at low frequencies is more sensitive to ear canal pressure manipulations than ER at high frequencies. For example, a 20 daPa change in ear canal pressure can produce a 10% shift in ER at 0.5 kHz whereas a 100 daPa shift may be needed around 3 kHz to exert the same effect. A non-linear relationship exists.
between ear canal pressure and change in ER. As ear canal pressure increasingly deviates from ambient pressure, further ER changes become increasingly smaller.

Figure 1. Mean energy reflectance in normal adult ears as a function of frequency for ambient pressure. Error bars represent one standard deviation from the mean.
Test-Retest Analysis

Mean data at half-octave frequency intervals were selected for conducting immediate test-retest analyses both without and with probe removal and reinsertion. Comparisons were made between steps 2 and 3 and between steps 3 and 4. Pearson correlation coefficients were calculated for each of the aforementioned measurement pairs at each half-octave frequency. Correlation coefficients for each frequency are displayed within the upper-left corner of each scatter plot shown in Figures 3 and 4.

Figure 2. Mean dynamic energy reflectance in normal adult ears (n = 46) as a function of frequency and ear canal pressure.
Figure 3. Immediate test-retest analysis of energy reflectance at ambient pressure in normal adult ears. Scatter plots for half-octave frequencies are displayed with Pearson correlation coefficients located in the upper-left corner for each individual panel.
Figure 4. Test-retest analysis of energy reflectance at ambient pressure following self-induction and equilibration of negative middle ear pressure (TPP = -40 to -65 daPa). Scatter plots for half-octave frequencies are displayed Pearson correlation coefficients located in the upper-left corner for each individual panel.
Figure 3 displays scatter plots for immediate repeated measurements without probe removal. Data from step 3 are plotted on the x-axes and data from step 4 on the y-axes. Correlations between test and retest were high at all frequencies with \( r \geq 0.95 \). Examination of correlations and scatter plots across frequency reveals no frequency-dependent trends with each plot depicting data points clustered tightly around their respective linear regression line.

Immediate test-retest comparisons were also made for aER measurements where the probe was removed and then reinserted into the ear. Figure 4 displays scatter plots of test-retest data with the first baseline measurement on the x-axis (step 2) and the retest measurement following probe removal and reinsertion on the y-axis (step 3). Correlation coefficients for frequencies between 0.25 and 2 kHz ranged from \( r = 0.63 \) to 0.78 with no apparent relationship between strength of correlation and frequency. For the highest test frequencies, 3 to 8 kHz, correlations were higher \( (r = 0.85 \text{ to } 0.9) \) than those found at lower frequencies. When comparing data from test-retest measurements without probe removal to those with probe removal, correlation coefficients were lower for all frequencies when the probe was removed and reinserted. The reduction in correlation coefficients for the probe removal condition was most pronounced at low- and mid-frequencies. Although correlation coefficients were also reduced at high-frequencies, it was to a lesser degree compared to low- and mid-frequencies.

Effects of Sweeping Pressure on Energy Reflectance Measurements

To determine if the manipulation of sweeping ear canal pressure resulted in differences in measured ER, a comparison was made between aER measurements and dER measurements in
ears with normal MEP as well as in ears with negative MEP. The sample size in the former comparison was reduced to 46 ears; two ears had to be excluded due to data file corruption. The latter comparison was made using ears with self-induced negative MEP between -40 and -65 daPa (n = 17). Differences in sample size stem from the inability of some participants to create and maintain a negative MEP for the duration of the measurement session. This set of comparisons allows for the examination of any alterations in ER resulting from sweeping pressure effects as well as detection of any interaction effect between sweeping ear canal pressure and MEP.

Data from steps 1 and 2 were used to compare ER measured with ambient and dynamic pressure methods. Since dER measurements were made with ear canal pressures swept from +200 to -300 daPa, spreadsheet filters were used to extract data points corresponding to the ear canal pressure equal to 0 daPa. This should be nearly equivalent to measurement at ambient pressure. Mean ER ratio as a function of frequency for both aER and dER data are displayed in Figure 5a for 46 normal ears; error bars represent one standard deviation from the mean. Error bars were plotted in only one direction for each curve in order to maintain clarity within the figure. Differences between the two means are shown in Figure 5b with negative values indicating that ER was greater for the aER measurement. Asterisks have been placed adjacent to those frequencies (half-octaves) where significant mean differences were found at the p < 0.01 level. From 1.5 to 3 kHz, ER was significantly lower when measured with sweeping ear canal pressure. The largest difference occurred at 2.119 kHz where ER was about 6% less in the dER (re: aER) measurement.
Figure 5. (a) Mean energy reflectance as a function of frequency for ambient and dynamic (ear canal pressure = 0 daPa) pressure measurements in ears with normal middle ear pressure. Error bars represent one standard deviation. (b) Mean difference between ambient and dynamic pressure energy reflectance measurements. Asterisks indicate significant differences (p < 0.01).
Figure 6. (a) Mean energy reflectance as a function of frequency for ambient and dynamic (ear canal pressure = 0 daPa) measurements in ears with negative middle ear pressure (TPP = -40 to -65 daPa). Error bars represent one standard deviation. (b) Mean difference between ambient and dynamic pressure energy reflectance measurements. Asterisks indicate significant differences (p < 0.01).
For the same comparison in ears with negative MEP, data was used from steps 6 and 7. Figure 6a displays mean ER as a function of frequency for aER and dER with unidirectional error bars representing one standard deviation from the mean. Figure 6b illustrates the differences between aER and dER measurements. Overall, mean differences were small with the largest change evinced as an ER increase of less than 3% at 1 kHz in the dER condition (re:aER). Paired t-tests performed at half-octave frequencies revealed significant differences at 0.75, 1, 6, and 8 kHz.

Effects of Ear Canal and Middle Ear Pressure Manipulations on Energy Reflectance

Measurements of aER were made immediately before self-induction (step 4) and immediately after equilibration of negative MEP (step 9). The first set of data from all 35 subjects able to create a negative MEP was used for this analysis. Comparison of means were made to determine if pressure manipulations resulted in any residual effects on ER, even after MEP had returned to near its baseline pressure. Figure 7 displays mean differences as a function of frequency. ER above the zero axis indicates that pressure manipulations resulted in increased ER. Conversely, ER below the zero axis is consistent with decreased ER following pressure manipulations. The largest mean differences were ER reductions less than 7% in the 4 to 5 kHz region. Paired t-tests performed at half-octave frequencies revealed significantly less ER following pressure manipulations at 0.25, 0.375, and 0.5 kHz ($p < 0.01$). Mean differences at these frequencies were always less than 4%. 

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Effects of Negative Middle Ear Pressure on Energy Reflectance

Data obtained from steps 3 and 7 were analyzed from participants able to self-induce and maintain a negative MEP. Data obtained from each measurement session were placed into one of four groupings based on MEP: -40 to -65 daPa, -70 to -95 daPa, -100 to -125 daPa, and < -125 daPa. For those participants who were able to create various MEPs, multiple datasets from the same ear were analyzed if each MEP did not fall within the same pressure grouping as another. This was not viewed as a problem since statistical comparisons were not performed across MEP groupings.

Figure 7. Mean difference between ambient pressure energy reflectance measurements recorded immediately before self-induced alterations in middle ear pressure and immediately after equilibration of negative middle ear pressure. Asterisks indicate significant differences (p < 0.01).
Mean aER as a function of frequency in both normal and negative MEP conditions are displayed in Fig 8. For all MEP ranges mean ER ratios are high from 0.2227 to 0.4204 kHz, ranging from 0.91 to 0.98. As frequency increases, ER ratio declines for all MEP ranges with ER ratio minimums occurring between 2.997 and 3.776 kHz. ER ratio minimums range from 0.18 in the -40 to -65 daPa group to 0.28 in the two groups with the most negative MEP ranges. Above the minimum, ER ratio increases with increasing frequency until it reaches approximately 0.7 to 0.8 at around 6 kHz or higher.

Figure 8. Mean energy reflectance for ambient pressure measurement as a function of frequency for ears with normal and negative middle ear pressure. Asterisks indicate significant difference ($p < 0.01$). Each panel represents a middle ear pressure range indicated above the panel.
Negative MEP resulted in frequency-dependent changes in ER ratio. Figure 9 displays ER change (negative MEP – normal MEP) as a function of frequency. The smallest changes in ER occurred with the smallest shifts in MEP (TPP = -40 to -65 daPa, red line). ER ratio increased for frequencies below 1.8 kHz with ER change becoming larger with increasing frequency up to 1.06 kHz. ER change decreased with increasing frequency through 1.888 kHz where ER change became independent of frequency through 2.828 kHz. From 2.997 to 5.657 kHz ER decreased under negative MEP but at the highest frequencies, 5.997 to 8 kHz, ER increased. Paired t-tests revealed that mean aER in the negative MEP condition was significantly higher than the normal MEP condition from 0.5 to 1.5 kHz and significantly lower at 4 kHz ($p < 0.01$). Figure 8 displays asterisks at frequencies where significant differences were found.

ER in ears with negative MEP between -70 and -95 daPa (Figure 9, green line) increased below 3 kHz. This represents a wider range of affected frequencies compared to the -40 to -65 daPa group. Larger decreases in ER at high-frequencies were present, especially between 4 and 6 kHz. For this MEP range, ER did not demonstrate an increase at the highest test frequencies relative to the normal MEP range.
The largest shifts in MEP (Figure 9, blue and violet lines) resulted in increases in ER below 2.9 kHz with reductions always larger than 30% between 1 and 1.5 kHz. Minimal change occurred near 3 kHz with decreases in ER above 4 kHz. Decreases in ER at high-frequencies were largest between 5 and 6 kHz and always exceeded 20%.

A quantitative examination of the effect of negative MEP on ER as a function of frequency (Figure 9) was performed in order to delineate systematic trends across MEP groups. Increases in ER at lower-frequencies and decreases in ER at higher-frequencies were analyzed, respectively, for each MEP range. Two parameters were used to aid in describing changes in ER due to negative MEP. First, the bandwidth for the range of frequencies in which ER either

\[ \text{Figure 9. Mean change in energy reflectance for ambient pressure as a function of frequency for four middle ear pressure ranges.} \]
increased or decreased by a set amount was determined and referred to as BW∆x where BW refers to frequency bandwidth and ∆x refers to percentage change in ER. The BW∆+20 refers to the arithmetic difference between the highest and lowest contiguous frequencies displaying a 20% increase in ER. Conversely, BW∆-10 represents the arithmetic difference between the highest and lowest contiguous frequencies displaying a 10% decrease in ER. The x value in BW∆x was chosen based on exploratory graphical analysis but, admittedly, the choice was arbitrary. Second, the frequencies at which the largest ER increase and decrease occurred are referred to as the peak frequency (f_peak) and the dip frequency (f_dip), respectively. With these parameters, the ER change was quantitatively analyzed for the four MEP groups (Table 1).

**Table 1**

*Characteristic Changes in Energy Reflectance (ER) Resulting from Negative Middle Ear Pressure (MEP)*

<table>
<thead>
<tr>
<th>MEP Group</th>
<th>ER Increase</th>
<th>ER Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW∆+20 (kHz)</td>
<td>f_peak (kHz) (Δx)</td>
</tr>
<tr>
<td>-40 to -65 daPa</td>
<td>0.5858</td>
<td>1.06 (+26%)</td>
</tr>
<tr>
<td>-70 to -95 daPa</td>
<td>0.6201</td>
<td>1.06 (+28%)</td>
</tr>
<tr>
<td>-100 to -125 daPa</td>
<td>1.047</td>
<td>1.414 (+37%)</td>
</tr>
<tr>
<td>&lt; -125 daPa</td>
<td>1.412</td>
<td>1.414 (+42%)</td>
</tr>
</tbody>
</table>
The group creating the smallest shift in MEP (-40 to -65 daPa), displayed their largest increase in ER from 0.7492 to 1.335 kHz (BWΔ+20 = 0.5858 kHz); all frequencies increased by at least 20% relative to ears with normal MEP. For the same group the $f_{\text{peak}}$ occurred at 1.06 kHz where ER increased by nearly 26%. For the -70 to -95 daPa group the BWΔ+20 increased to 0.6201 kHz due to an increase in the upper bound frequency to 1.414 kHz. However, the $f_{\text{peak}}$ remained unchanged at 1.06 kHz where magnitude of ER enhancement grew only slightly (28%). Larger negative shifts in MEP represented by the -100 to -125 daPa group were associated with a larger BWΔ+20. Although the lower bound frequency shifted upward by only a small amount (to 0.8409 kHz), the upper bound frequency evinced a larger shift to 1.888 kHz. This resulted in a BWΔ+20 equal to 1.047 kHz. The $f_{\text{peak}}$ shifted upward to 1.414 kHz where a 37% increase in reflectance occurred. The systematic increase in BWΔ+20 continued for the less than -125 daPa group; its bandwidth was extended to approximately one and one-half octaves (from 0.7071 to 2.119 kHz). This resulted in the largest BWΔ+20 which equaled 1.412 kHz. Yet the $f_{\text{peak}}$ did not shift upward relative to the -100 to -125 daPa group, but ER change at the $f_{\text{peak}}$ grew to 42%.

The increase in ER from around 0.5 to 1.5 kHz represented the major change due to negative MEP, but a substantial decrease in ER also occurred at high-frequencies starting at around 3 to 4 kHz. For the -40 to -65 daPa group, the BWΔ-10 was 0.802 kHz with lower bound and upper bound frequencies of 4.238 and 5.04 kHz, respectively. The $f_{\text{ap}}$ for this pressure grouping was 4.49 kHz with a corresponding ER decrease of nearly 12%. With larger negative MEP shifts within the -70 to -95 daPa range the BWΔ-10 increased to 1.101 kHz with the wider bandwidth attributable to an increase in the upper bound frequency to 5.339 kHz. The $f_{\text{ap}}$
remained stable at 4.49 kHz yet produced a greater ER reduction effect (18%). For the two pressure groups containing ears with the largest pressure shifts, -100 to -125 daPa and < -125 daPa groups, the BW\(\Delta\)-10 increased to 2.489 and 2.637 kHz, respectively. These changes were accompanied by upward shifts in \(f_{dip}\) to 5.04 kHz (ER decline of 22%) and 5.339 kHz (ER decline of 25%) for the -100 to -125 daPa and < -125 daPa groups, respectively. In summary, negative MEP resulted in ER increases at low- and mid-frequencies as well as ER decreases at high-frequencies. These changes with decreasing MEP were expressed, quantitatively, by increases in BW\(\Delta\)+20, BW\(\Delta\)-10, \(f_{peak}\), and \(f_{dip}\).

To further illustrate the effects of negative MEP on ER, the ER ratio is shown as a function of MEP (Figure 10). Data were filtered and files selected so that each participant contributed a single set of data for the linear regression analyses. All 48 subjects were included. For those subjects who created multiple negative MEPs, data from the largest shift in MEP was chosen. This method was chosen in an attempt to achieve an equitable number of subjects in each of the four negative MEP groups. Because this method alone did not achieve equity between groups, two subjects were moved to make 8 subjects in each negative MEP group. The normal MEP group was represented by the 12 subjects who were not able to create a negative MEP. Admittedly this approach was biased but it did provide equal numbers across negative MEP groups and ensure that each subject was represented by a single set of MEP data. Moreover, the extant literature on Eustachian tube function does not provide support for a strong association between typical middle ear function and the ability to self-induce a negative MEP thus reducing concerns regarding subject selection bias (Falk, 1981; Riedel, Wiley & Block, 1987). Subjects in
the normal MEP group with small positive tympanometric peak pressures (e.g., +5 or +10 daPa) were represented within the regression analysis by MEPs of the same pressure magnitude but opposite polarity. This was done so that small positive pressures did not artificially deflate correlation coefficients. This data manipulation is thought to have a small effect on data analyses due to the small magnitudes of MEP and can be justified by the similar, although not identical, effects of positive and negative MEP.

Linear regression analyses were performed at standard audiometric frequencies (0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 kHz). From 0.25 to 1 kHz, correlations were negative and strength of correlation increased with increasing frequency ($r = -0.39$ to -0.74). From 1.5 to 3 kHz, strength of correlation decreased with increasing frequency ($r = -0.67$ to -0.14). As frequency further increased from 4 to 6 kHz, a reversal in the direction of correlation occurred ($r = 0.27$ to 0.38). At 8 kHz, ER was independent of MEP. Overall, the strongest correlations were negative and found at 1 kHz ($r = -0.74$) and 1.5 kHz ($r = -0.67$).

The effects of negative MEP on ER can be explored across a wide range of ear canal pressures by examining data from dER measurements. The -40 to -65 daPa group was selected because it had the largest sample size (n = 17) of the four negative MEP groups. ER in the baseline condition was subtracted from the negative MEP condition. Mean differences are displayed as a contourplot in Figure 11. When ear canal pressure was near ambient pressure (-25 to 25 daPa), negative MEP caused the largest ER change for frequencies between 0.6 and 1.5 kHz. ER tended to increase by 15 to 25% with negative MEP of -40 to -65 daPa. When ear canal pressure was more positive than +100 daPa or more negative than -150 daPa, the effects of
negative MEP on ER between 0.6 and 1.5 kHz were reduced to increases or decreases no greater
than 5 to 10%, respectively. High-frequencies were less affected by negative MEP with small
decreases (~10%) in ER around 4 to 5 kHz. Frequencies above 6 kHz were relatively insensitive
to changes in MEP and ear canal pressure. For example, the 5% increase in ER around 7 kHz due
to negative MEP is effected only minimally by changes in ear canal pressure.
Figure 10. Energy reflectance ratios for ambient pressure measurement as a function of negative middle ear pressure. Each panel represents a single frequency with Pearson correlation coefficients embedded in panel corners (n = 48 ears).
Energy Reflectance with Compensated Negative Middle Ear Pressure

The effectiveness of compensation for negative MEPs was examined by comparing and contrasting data from steps 3, 6, and 7. For each MEP group ER comparisons were made in the same ears under three conditions: normal MEP, negative MEP, and compensated MEP. Compensated MEP data were derived by selecting data points from the dER measurement (step 6) where the positive ear canal pressure equaled the difference in TPP obtained under negative MEP and baseline conditions. In other words, a compensated MEP is a condition in which applied positive ear canal pressure was equal to the negative shift in MEP self-induced by a subject.

Figure 11. Mean difference in energy reflectance as a function of frequency and ear canal pressure between normal middle ear pressure and negative middle ear pressure (TPP = -40 to -65 daPa). Positive values indicate an increase in reflectance with negative middle ear pressure whereas negative values indicate a decrease in reflectance.

Energy Reflectance with Compensated Negative Middle Ear Pressure

The effectiveness of compensation for negative MEPs was examined by comparing and contrasting data from steps 3, 6, and 7. For each MEP group ER comparisons were made in the same ears under three conditions: normal MEP, negative MEP, and compensated MEP. Compensated MEP data were derived by selecting data points from the dER measurement (step 6) where the positive ear canal pressure equaled the difference in TPP obtained under negative MEP and baseline conditions. In other words, a compensated MEP is a condition in which applied positive ear canal pressure was equal to the negative shift in MEP self-induced by a subject.
Negative MEP compensation was deemed complete if ER in the compensated MEP condition did not differ substantially from ER in the normal MEP condition. ER as a function of frequency is shown in Figure 12 for three conditions: normal MEP, uncompensated negative MEP, and compensated negative MEP. Mean ER ratios as a function of frequency for compensated negative MEP relative to uncompensated negative MEP and normal MEP are shown in Figure 13. ER below 0 indicates that ER in the compensated negative MEP condition was lower than the comparison condition (normal MEP or negative MEP). ER above 0 indicates that ER in the compensated negative MEP condition was greater than the comparison condition. For most frequencies, ER with compensated MEP was lower than ER with normal MEP. These differences were small (never exceeding a 10% change) and independent of frequency. Mean ER change averaged across all frequencies was 5% for all pressure ranges except -100 to -125 daPa where it was only 3%. Comparison of ER means, in each of the negative MEP groups, using t-tests at half-octaves revealed that significant differences were infrequent and unsystematic in occurrence (Figure 13).
In contrast, a comparison of compensated negative MEP with uncompensated negative MEP conditions revealed significant differences across a wide range of frequencies for the four negative MEP ranges. The pattern of mean differences found was similar to that reported with aER under normal MEP versus negative MEP conditions. However, at low- and mid-frequencies ER increases found in the present comparison were slightly larger and high-frequency decreases slightly smaller above 4 kHz. Specifically, ER with compensated negative MEP was significantly lower than ER with negative MEP \((p < 0.01)\) at all half-octave frequencies from 0.25 to 1.5 kHz across all MEP ranges. ER under compensated negative MEP was also significantly lower from 2

Figure 12. Mean energy reflectance as a function of frequency for ears with normal, negative, and compensated middle ear pressure. Each panel contains a different middle ear pressure range indicated by the panel title.
to 3 kHz for the < -125 daPa MEP range ($p < 0.01$). Significant decreases in ER were found only at 6 kHz and were present for all four MEP ranges ($p < 0.01$).

![Graphs showing the change in energy reflectance as a function of frequency between compensated middle ear pressure and normal or negative middle ear pressure, with asterisks indicating significant difference ($p < 0.01$). Each panel contains a different middle ear pressure range indicated by the panel title.](image)

Figure 13. Mean difference in energy reflectance as a function of frequency between compensated middle ear pressure and normal or negative middle ear pressure. Asterisks indicate significant difference ($p < 0.01$). Each panel contains a different middle ear pressure range indicated by the panel title.
Normative Energy Reflectance Measurements

The present study demonstrated ambient pressure energy reflectance (aER) measured in 48 ears with normal middle ear function. The general trend of aER versus frequency function in the human middle ear was that of high energy reflectance (ER) at low frequencies, monotonically decreasing as function of frequency. A minimum in the ER versus frequency function occurred between 1 and 4 kHz. This decline in ER represents a decreasing impedance mismatch with increasing frequency up to around 1 kHz (Allen et al., 2005). Above around 4 kHz, the impedance mismatch increases as evidenced by the increase in ER (Feeney & Sanford, 2004; Keefe et al., 1993; Margolis et al., 1999; Shahnaz & Bork, 2006; Voss & Allen, 1994). In Figure 14, mean data for ears with normal middle ear pressure (MEP) is plotted alongside mean data from two previous studies. The two studies chosen for comparison were selected because mean data were readily available in published journal articles and each uses one of the two ER systems most often used in this line of research. The Feeney and Sanford (2004) study used an the ReflWin system developed by Dr. Keefe at Boy's Town National Research Hospital. Shahnaz and Bork (2006) utilized the commercially-available system developed by Mimosa Acoustics. The test system used in the present study is a more recent version of the ReflWin system developed in collaboration with Interacoustics and described in detail by Liu and colleagues (2008).
In Figure 14, the ER versus frequency function displays greater fine structure in the present study compared with mean data from the two comparison studies. This is due to the finer frequency resolution chosen for analysis in the present study. Close agreement between mean ER data from the present study and from Shahnaz and Bork (2006) is apparent for frequencies up to around 3.2 kHz. Data from Feeney and Sanford (2004) demonstrated higher ER at low- and mid-frequencies. The monotonic rise in ER with increasing frequency occurred in all three studies but with differences in the starting frequency. In the present study, ER rose with increasing frequency above around 3.2 kHz whereas the starting frequency was higher (~ 4 kHz) for the two other research groups.

Figure 14. Mean energy reflectance as a function of frequency. Data displayed from the present study as well as data from two other research groups.
comparison studies. The reason for these differences is not clear but may be related to differences in probes, probe tips, calibration, or subject samples. Data from the present study also demonstrated a rollover effect at the highest frequencies where ER decreases above 6.6 kHz. Most studies in recent years, including the two comparison studies in Figure 14, have not reported data out to such high frequencies. However, a recent study by Liu et al. (2008), as well as older reports by Hudde (1983) and Stinson (1990), have found similar ER declines at high-frequencies.

**Test-Retest Reliability**

The present study demonstrated that correlation coefficients for immediate test-retest aER measurements, without changes in probe position, were very high ($r > 0.95$) for all frequencies tested. These findings are consistent with a strong positive correlation between test and retest measurements indicating that individual ears maintained their positions within the sample for repeated measurements. This type of strong covariant relationship is requisite, but not sufficient, for establishing satisfactory test-retest reliability. Mean ER differences between the two repeated tests were less than 1% at all frequencies tested. This was an expected finding considering the negligible changes in probe position and time between measurements. The combination of signal averaging as well as the high signal-to-ratios found in ER measurements were expected to minimize the variation due to environmental and physiological noise, even at very low test frequencies. For aER measurements made with a similar inter-test time interval but with probe removal and reinsertion, lower correlations were found. This was especially true for low- and
mid-frequencies. However, mean ER change was no greater than 5% at any frequency.

Vander Werff, Prieve, and Georgantas (2007) reported test-retest reliability data from 10 adult ears both with and without probe removal and reinsertion. They found that test-retest changes of less than 5% occurred across the entire test frequency range, irrespective of probe removal and reinsertion. Werner, Levi, and Keefe (2010) reported on test-retest reliability from up to 136 adult ears measured 2 weeks apart. Data were presented for third-octave bands from 0.281 to 7.336 kHz but due to exclusion of data points that indicated a negative resistance, the sample size across frequencies was not equal with many ears excluded at the lowest and highest frequencies. Their highest reported Pearson correlation coefficient was $r = 0.95$ at 0.365 kHz with a sample of 101 ears. Between 0.578 and 2.311 kHz test-retest correlations ranged from $r = 0.43$ to 0.69. For higher frequencies all correlations were less than $r = 0.4$. They also found that correlation coefficients were poorest at frequencies greater than or equal to 2.911 kHz. Inspection of the 90th percentiles from the adult subjects in Vander Werff et al. (2007) reveals that the greatest increase in variability with probe removal and reinsertion occurred above 2.5 kHz. These findings are in contrast to what was found in the present study where the highest test-retest correlations occurred at high-frequencies. One possible explanation for these differences may be related to the different probe tips used in these studies.

The present study utilized a rubber probe tip supplied with the Interacoustics/RefWin test system; whereas, Werner et al. (2010) used foam probe tips with an older version of the RefWin system and Vander Werff et al. (2007) used foam probe tips (in adult subjects) with the Mimosa Acoustics system. Data reported in Vander Werff et al. (2007) compared reliability of 11
infants measured with rubber probe tips and 10 infants measured with foam probe tips. They reported larger variability with probe removal and reinsertion for rubber probe tips than foam probe tips for frequencies below 0.5 kHz as well as at mid-frequencies. In contrast, their 90th percentiles indicated less variability with the rubber tips than the foam tips for high-frequencies. The prototype Interacoustics/ReflWin system used in this study uses rubber probe tips due to the need to obtain a hermetic seal for dynamic pressure ER measurements (dER); older ReflWin systems used the foam probe tips. The Mimosa Acoustics system offers the choice of either probe tip. It is possible that each type of probe tip may be best suited for particular measurement paradigms (e.g., foam probe tip for aER measurements and rubber probe tips for dER measurements). Further study is needed to optimize the acoustic coupling between the test probe and the ear.

Effects of Sweeping-Pressure on Energy Reflectance Measurements

Most previous studies measuring ER in human ears have performed aER measurements. Although a few investigations have reported ER measured at various positive and negative static ear canal pressures (Keefe & Simmons, 2003; Margolis et al., 1999; Margolis et al., 2001; Sanford & Feeney, 2008), it has not been until recently that it was possible to obtain this data with sweeping ear canal pressure (i.e., dER) in a manner similar to that of conventional tympanometry. The effects of sweeping ear canal pressure on aural acoustic admittance have been well documented for more than 25 years. This is an important consideration when interpreting ER measurements because of the close relationship between the physical parameters
of energy reflectance and admittance/impedance. Three forms of tympanometric changes have been linked to sweeping pressure measurements: increasing acoustic admittance with increasing sweep-pressure rate (e.g., Feldman, Fria, Palfrey & Dellecker, 1984), increasing separation between TPP and MEP with increasing sweep-pressure rate (Gaihede et al., 2005; Gaihede et al., 2000), and increasing acoustic admittance with increasing number of tympanometric measurements (Gaihede, 1996). Each of these factors could potentially contribute to differences between aER and dER measurements, but it is the last factor that is most pertinent to the present comparison.

For ears with normal MEP, frequencies between 1.5 and 3 kHz were significantly lower for dER measurements. Calculation of mean differences revealed that the largest discrepancy between the two measurements was small—a 6% difference occurring at 2.119 kHz. In the negative MEP group (TPP = -40 to -65 daPa), sweeping pressure resulted in higher ER ratios from 0.75 to 1 kHz and lower ER ratios from 6 to 8 kHz. The largest difference between these two conditions was also small, less than 3%. Liu et al. (2008) performed a similar comparison but measurements were reported in energy absorbance rather than ER. For clarity, their results were converted to, and reported as ER. Comparison between aER and dER measurements was made on 48 normal adults (both ears). Sweeping pressure resulted in lower ER at low-frequencies and slightly higher ER at high-frequencies. Their largest mean differences in each direction were a 16% decrease in ER at 0.84 kHz and a 6% increase at 4.76 kHz. Liu et al. suggested that their observed changes may be related to the formation of a small positive ear canal pressure between the probe tip and tympanic membrane resulting from probe insertion.
prior to the aER measurement.

For sweeping pressure measurements, the present study evinced a slight decrease in ER between 0.75 and 3 kHz. The largest mean differences occurred within different frequency regions and were smaller in magnitude than what was reported by Liu and colleagues (2008). In the present study, the largest mean difference was 6% at 2.119 kHz compared to a 16% difference at 0.84 kHz in Liu et al. The greater high-frequency ER in the dER condition reported by Liu et al. was not observed in the present study. Data from the negative MEP group (-40 to -65 daPa) in the present study evinced changes no greater than 3%. These differences occurred in the opposite direction to what would be predicted if a residual positive ear canal pressure were created. The smaller changes in the negative MEP group may be explained by the reduced sensitivity to ER changes by ear canal pressure variations (see Figure 2 and Figure 11c). Differences between studies may be related to the diameter or style (flanged vs domed) of the selected probe tip and/or probe insertion technique used by the tester. If the smaller probe tip was inserted more deeply, while still maintaining an adequate hermetic seal, a greater positive pressure may have formed.

The methodological design of the present study did not allow for delineation of the specific underlying mechanism(s) for the observed differences due to sweeping ear canal pressure. The effects of sweeping pressure rate are expected to be small due to the slow rate of pressure change (75 daPa/s). One possibility is that the small ER decreases in the normal MEP group due to sweeping pressure may be related to middle ear hysteresis caused by the viscoelastic properties of the middle ear. More information is needed to better understand this phenomenon.
and to determine the clinical significance of its effects. Future investigations should further investigate the effects of probe insertion technique, sweeping pressure rate, and sweeping pressure direction.

Effects of Ear Canal and Middle Ear Pressure Manipulations on Energy Reflectance

Based on a previous study from this laboratory using distortion product otoacoustic emission measurements (Sun & Shaver, 2009), ER ratios prior to pressure manipulations were not expected to be different from ER ratios following equilibration of MEP. However, this could not be ruled out *a priori*; the temporal effects of pressure manipulations on the middle ear system have not been well-studied. For example, following equilibration of negative MEP it is unclear how long it takes for the ossicular chain to return to its normal position (Hüttenbrink, 1988).

Results from the present study indicate that ER following pressure manipulations is reduced at low- and high-frequencies but increased at mid-frequencies. However, significant differences were found only at low-frequencies where ER was reduced compared to the baseline condition. In spite of these significant differences, mean differences due to pressure manipulations only slightly exceeded mean test-retest differences suggesting that these changes are not a major clinical concern.

Effects of Negative Middle Ear Pressure on Energy Reflectance

The present study was the first to utilize a within-subjects design to demonstrate the frequency-specific effects of negative MEP on ER measurements. Effects have been reported
across a wide frequency bandwidth and MEP range. In general, negative MEP resulted in larger ER increases for low- and mid-frequencies and smaller ER decreases at high-frequencies (see Figure 9). As negative MEP increased, the frequency bandwidth where ER changes occurred widened for both ER increases and decreases (see Table 1). Also, the frequency at which the largest changes occurred shifted towards a higher frequency.

In an early attempt to develop a clinical dER test (wideband tympanometry), Margolis et al. (1999) described ER measurements for normal adult ears made at various static ear canal pressures. ER measured at positive ear canal pressures is used for comparison because it is most comparable to negative MEP due to the similarity in tympanic membrane position. Numerical data were not available within this research report and could not be obtained from the author. Therefore, the g3data analyzer software (Frantz, 2000) was used to estimate values from the relevant graph (Figure 6 in Margolis et al., 2001). This software has been shown to provide acceptable accuracy for recovering data from scanned images of various quality (Bauer & Reynolds, 2008).

For an applied ear canal pressure of +56 daPa ER increased by up to 20% near 1 kHz, relative to ER measured at ambient pressure (Margolis et al., 2001). Above around 1 kHz the ER increase declined with increasing frequency through around 2.2 kHz where no change was present. As frequency increased further, ER decreased relative to the ambient pressure condition. The largest ER decrease was around 11% and occurred near 2.85 kHz. Similar ER reductions may have occurred at higher frequencies but this was difficult to ascertain due to the considerable overlapping of various plots within the figure from which data was extracted. Data
from Margolis et al. (2001) were also examined for the +100 daPa applied ear canal pressure condition. The frequency at which greatest ER enhancement occurred shifted upward to around 1.1 kHz where ER magnitude increased to around 24%. The lowest frequency to intersect with the zero axis increased to around 2.5 kHz. The largest decrease in ER was on the order of 18% and occurred around 4.5 kHz.

Sanford and Feeney (2008) performed ER measurements at static ear canal pressures, ranging from +200 to -200 daPa, on 20 normal adults. Application of +50 daPa ear canal pressure resulted in an ER was increase at low- and mid-frequencies with maximum increase around 17% near 1 kHz. As frequency increased further, ER change decreased until no change was present at around 2.2 kHz. As frequency increased above 2.2 kHz, ER decreased (re: baseline) up to around 6% near 2.5 kHz. For application of ear canal pressure equal to +100 daPa, ER changes were larger in magnitude with an increase of approximately 25% at 1 kHz. Above around 1.5 kHz, ER change decreased until it crossed the zero axis at around 3 kHz and reached an ER decrease of around 4% near 3.5 kHz.

Findings from these studies using positive ear canal pressure are similar to results from the present study but with somewhat smaller effects for some conditions. For both the two comparison studies and the present study, application of positive ear canal pressure (+56 or +50 daPa) or creation of a negative MEP (-40 to -65 daPa) resulted in maximum ER increase around 1 kHz. However, greater changes occurred under negative MEP (26%) compared to positive ear canal pressure (17 to 20%). At high-frequencies ER reductions were as high as 12% in the present study and 11% in Margolis et al (2001). Reduction magnitude did not exceed around 6%
in Sanford and Feeney (2008) but this may be related to a reduced high-frequency test range. With +100 daPa of ear canal pressure or negative MEP between -70 and -95 daPa, maximal ER increase across studies occurred around 1 kHz. Magnitude of change was consistent across studies with ER increases ranging from 24 to 26%. For both the present study and Margolis et al., maximum ER decrease was around 18% and occurred at 4.5 kHz. Results from Sanford and Feeney were not comparable, likely owing to the reduced high-frequency range. Overall, data from subjects with experimentally-induced negative MEP has been shown to be quite similar to data from ears with an equivalent amount of positive ear canal pressure. This generalization is limited to small negative MEPs in young adults without significant history of middle ear pathology.

The ER measurement has never before been investigated in human ears with experimentally produced negative MEP. Although a few studies have reported ER from individual patients or groups of children with naturally-occurring negative MEP, it is difficult to quantify the effects of negative MEP due to the observational nature of their research designs. Hunter, Tubaugh, Jackson & Propes (2008) tested aER in infants and young children and compared 124 ears with normal MEP to 9 ears with mild negative MEP (TPP < -100 daPa). Their data revealed that average ER was higher in the negative MEP group (re: normal MEP group) for all frequencies up to around 4 kHz. Beers and colleagues (2010) reported mean aER for children in ears with differing degrees of negative MEP. Test subjects were separated into three groups: 144 ears with normal MEP (TPP > -99 daPa), 30 ears with mild negative MEP (TPP = -100 to -199 daPa), and 24 ears with severe negative MEP (TPP = < -200 daPa). Below 6 kHz, ER was
lowest for the ears with normal MEP. However, the largest mean differences fell within the 0.4 to 1.8 kHz range where group differences were around 20% when ears with normal and severe negative MEP were compared. Mean ER increases, averaged across frequency, from 0.4 to 1.8 kHz were 20% for the -100 to -125 daPa group and 22% for the < -125 daPa. Despite the age differences (children vs. adult) and methodological differences (between-subject vs. within-subject comparisons) between studies, the magnitude of these negative MEP-related effects is consistent with previous reports.

However, not present in the data from either Beers et al. (2010) or Hunter, Tubaugh et al. (2008) was the high-frequency ER decrease around 5 kHz observed in the present study (see Figure 9). Similar high-frequency reductions in ER have been reported when positive ear canal pressure was applied to both adult and infant ears (Margolis et al., 2001; Sanford & Feeney, 2008). Data from the present study are in closer agreement with studies reporting ER from normal adults with positive ear canal pressure rather than studies reporting ER from children with naturally-occurring negative MEP. There are several possibilities for these differences. It is possible that a proportion of those children tested had middle ear effusion which was not detected by other tests. Alternatively, age-related differences may have resulted in the high-frequency ER decreases being shifted to higher frequencies which were beyond the upper limit of the measurement system. The use of a between-subjects design may have masked high-frequency changes in children. If the frequencies at which reductions occurred was related to age/head size, averaging across subjects may have obscured these differences. Further investigation of the effects of naturally-occurring negative MEP on ER should be studied across a wide range of ages.
using both aER and dER measurement techniques.

The relationship between negative MEP and ER ratio was determined at standard audiometric frequencies. Correlation coefficients ranged from as low as 0.02 (non-significant) at 8 kHz to -0.74 at 1 kHz. Inspection of linear regression lines in each of the scatter plots revealed an undulating pattern in the slopes of the regression lines. From 0.25 to 1 kHz, the slope of the linear regression line steepened with increasing frequency. For frequencies of 2 kHz and higher this pattern reversed and with a change in the direction of the relationship by 4 kHz. No relationship between MEP and ER was found at 8 kHz. Frequencies with the strongest relationship to negative MEP were 1 and 1.5 kHz where, respectively, 55% and 45% of the variance was explained for by MEP. Present findings suggest that MEP variations cannot accurately predict ER.

Energy Reflectance with Compensated Negative Middle Ear Pressure

The present study has shown that application of ear canal pressure equivalent to the magnitude of the negative MEP restores ER ratios to near their baseline values. This compensation was effective for nearly all frequencies and all negative MEP ranges. ER in the compensated negative MEP condition was significantly lower than in the uncompensated condition for 0.25 to 3 kHz for the < -125 daPa group and from 0.25 to 1.5 kHz for the other three groups. Significant increases in ER were also found at either 6 or 8 kHz, depending on the MEP group.

Mean ER ratios from the negative MEP condition were compared with those from the
compensated negative MEP condition to determine the effectiveness of using positive ear canal pressure to counteract the effects of negative MEP. Although 5 out of 44 paired t-test comparisons were significant ($p < 0.01$), residual differences between compensated ER and baseline ER were always less than 10%. The consistently lower ER under the compensated condition suggests the presence of systematic error. One possibility is that this effect may be attributable to the viscoelastic properties of the middle ear system. Gaihede (1996) has demonstrated that repeated pressure loading and unloading to the middle ear system resulted in increased compliance for each subsequent tympanometric measurement. An increased compliance would be equivalent to a decreased ER ratio in the compensated negative MEP condition. Further study is needed to explore the effects of sweeping ear canal pressure on ER measurements.

Residual differences between the normal MEP and compensated negative MEP conditions were small and, as a result of ear canal pressure compensation, ER was restored to near baseline values. The present findings suggest that ear canal pressure compensation may be useful in clinical applications of the ER test but further investigations in clinical populations are warranted. Hunter and Margolis (1997) described a clinical report of a 9-year-old child with history of recurrent otitis media but normal tympanometric findings except for a negative MEP of -263 daPa. When aER was measured at ambient pressure the results could not be easily interpreted due to the confounding effects of the negative pressure gradient. Following ear canal pressure compensation it was determined that ER was abnormal. Video otomicroscopy provided further evidence for the presence of otitis media.
Using a test protocol similar to that used in the present study, Sun and Shaver (2009) demonstrated that when subjects self-induced negative MEPs (ranging from TPP = -40 to -420 daPa) distortion product otoacoustic emission amplitudes were reduced in a frequency-dependent manner. Moreover, otoacoustic emission amplitudes were restored to near baseline values by applying an equivalent negative ear canal pressure. Clinical use of negative ear canal pressure compensation has demonstrated increases in transient evoked otoacoustic emission amplitudes and screening pass rates for a group of children from 1- to 7-years of age (Hof et al., 2005; Hof, Dijk, Chenault & Anteunis, 2005). Promising laboratory data from the present study, in conjunction with previously reported laboratory and clinical data, suggest the need for clinical studies to establish the effectiveness of the compensation procedure.

Summary

The purposes of this investigation were to establish test-retest reliability of the aER test, to determine the frequency-dependent effects of negative MEP on ER ratios, and to determine if ear canal pressure compensation could be used to restore ER ratios to near baseline levels. Immediate test-retest reliability was demonstrated for conditions in which the probe was fixed and also when it was removed and reinserted. Reliability for the without probe removal condition was excellent across frequency and reliability for the with probe removal condition was excellent at high-frequencies but only adequate at low-frequencies. Comparison of the present test-retest data to recent studies reporting reliability data suggests the possibility that probe tip style or insertion technique may have an effect on test-retest reliability. Future investigations should
establish the error contribution introduced by probe insertion technique as well as the suitability of each probe tip style for particular applications and populations.

The frequency-dependent effects of negative MEP on ER ratio were explored by teaching subjects to self-induce a negative MEP via performing a Toynbee maneuver or a closed-nostril sniffing procedure. Data from subjects able to create a negative MEP were placed into one of four groups: -40 to -65 daPa, -70 to -95 daPa, -100 to -125 daPa, and <-125 daPa. ER increases due to negative MEP occurred at all frequencies below 2.8 to 3.8 kHz with the cutoff frequency dependent on the degree of negative MEP. Below around 0.85 kHz, ER increases were unsystematic and seemingly unrelated to magnitude of negative MEP. Maximum ER increases always occurred in the 1 to 1.5 kHz frequency region with magnitudes around 25 to 40%. A point of no change occurred between 2.8 and 3.8 kHz. ER decreases always occurred at high-frequencies with maximum decreases around 10 to 20% near 4.5 to 5 kHz. Systematic changes in ER were shown to be related to the magnitude of negative MEP but linear regression analyses only provided modest evidence of this between 1 and 1.5 kHz. Strength of correlation was not improved using polynomial regression. Data from the present study are in reasonable agreement with previous studies using application of positive ear canal pressure to stiffen the middle ear system (Margolis et al., 2001; Sanford and Feeney, 2008). The present findings are also compatible with clinical studies of negative MEP in children demonstrating ER increases of around 20% in the low- to mid-frequencies (Beers et al., 2010). However, a discrepancy exists between studies; the high-frequency ER decrease found in the present study was not observed in previous clinical studies. Future studies on negative MEP are needed to compare effects in adults
versus children. Use of longitudinal designs should be prioritized.

    Application of a negative ear canal pressure, equivalent in magnitude to the negative
MEP, was found to restore ER ratios near their baseline values across all frequencies for all four
negative MEP ranges. Although restored ER ratios were systematically lower across all
frequencies and MEP ranges compared to the normal MEP condition, this difference never
exceeded 10%. Future investigations should investigate test performance in subjects with
combined negative MEP and otitis media to determine if compensation provides improved test
performance over the uncompensated condition.
REFERENCES
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