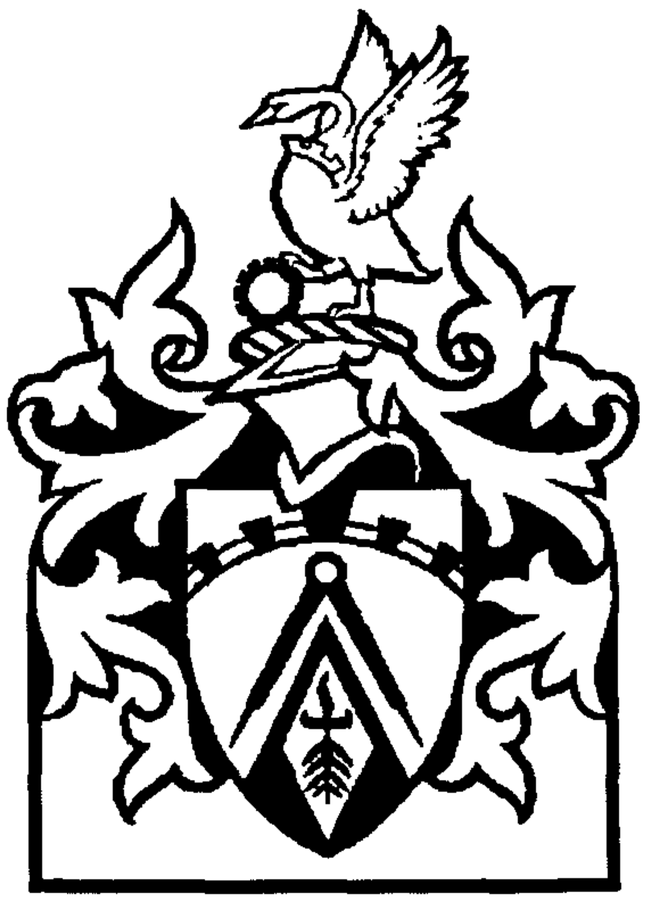

MATURATION OF AUDITORY PARAMETERS IN THE PRETERM INFANT WITH PARTICULAR REFERENCE TO DIET

A Thesis submitted for the degree of

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by

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ABSTRACT

This study was developed from the original protocol which was to examine the role of long chain polyunsaturated fatty acids (LCPUFA) in the maturation of the auditory system in the preterm infant. The particular dietary components under investigation were arachidonic (AA) and docosahexaneoic (DHA) acids.

This study investigates the wider aspects and implications of the preterm birth on the maturation of the preterm auditory system. Testing was performed using Auditory brainstem response (ABR) on preterm infants in Hillingdon Hospital's neonatal intensive care unit (NICU). The recruitment criteria was <32 weeks gestational age (GA) or <1500g. A normative data set was produced using term infants (37-42 weeks GA).

Tympanometric testing was also introduced to assess middle ear (ME) function in both preterm and term infants. The susceptance and conductance components were recorded at frequencies between 226 and 2000Hz. This testing enabled normative data to be established for multi-component, multi-frequency tympanometric analysis. The effect of the preterm birth on tympanometric data was also examined.

A total of 22 preterm infants were recruited onto the study. The maturational characteristics of ABR parameters relating to neural transmission and synaptic efficacy were investigated. The peripheral auditory system (PAS) and the lower brainstem region were found to have maturation in their neural transmission properties. The auditory nerve, however, displays constant neural transmission properties throughout the preterm period. Maturation of the synaptic efficacy (using the rate effect) of the PAS was not identified. However, both auditory nerve and lower brainstem regions display reductions in the rate effect. This was greatest in the auditory nerve.

The effect of the preterm birth was assessed. The PAS showed lesser conductive properties for the preterm infant during the term period. This behaviour was confirmed by poor tympanometric data for the preterm infants. The auditory nerve showed the same transmission properties for both term and preterm infants. This indicates that the lack of maturation in this region is replicating the characteristics of the term infant developing in utero. The lower brainstem region would seem to be the most susceptible to delayed development in the preterm infant. The rate effect for the PAS and auditory nerve indicate that the extra-uterine environment has not been detrimental to the maturation of synaptic efficacy as measured by the rate effect. The lower brainstem region rate effect is slightly higher for the preterm infants. This may indicate that this region is the most susceptible to possible synaptic deficiency.

There is indication of a gender effect for transmission properties in the preterm infant. This suggests superior properties for females in the central auditory system. It is possible that this also occurs on a synaptic level. Dietary analysis suggested that the preterm infants (on this study) fed formula milk enriched with a LCPUFA composition have comparable auditory function (as measured by ABR) with breastfed infants. It is concluded that breastmilk would be the diet of choice due to the additional medical benefits.

Tympanometry was found to be well tolerated in both term and preterm neonate populations. Testing in enclosed style incubators in the NICU was successful. Normative characteristics for multi-component tympanometry at various frequencies was established. The ear canal acoustic and mechanical properties were identified as a source of variability for tympanogram morphology in the neonate populations. The interaction of the neonate external auditory meatus (EAM) violates assumptions relating to numerical data. A lack of maturation was found for preterm data, there was no evolution to the normative term data over the period studied. The more complex nature of the preterm tympanometric data was still observed during the term period. This indicates that there is a difference between the maturational characteristics of the term infant and those for the preterm infant in the clinical environment.

The PAS ABR characteristics were compared with tympanometric data collected from the preterm infants. The lack of maturation in the tympanometric data suggests that the major contribution to the ABR maturation during the preterm period is due to the cochlear transduction and basic synaptic delay components. In addition, that poorer ME function (as displayed in the tympanometric data) contributes to the lesser PAS ABR conductive properties in the preterm population by the term period.

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LIST OF ABBREVIATIONS / NOMENCLATURE

AA..... arachidonic acid
ABR ..... auditory brainstem response
AEP..... auditory evoked potential
A-I..... amplitude-intensity (function)
ALA ..... α-linolenic acid
ANOVA ..... analysis of variance
B_a/B..... (acoustic) susceptance
ΔB ..... change in susceptance
CAP..... compound action potential
CE ..... cholesterol esters
C&G..... cow and gate limited
CNS..... central nervous system
dGa..... days gestational age
DHA..... docosahexaenoic acid
EBM..... expressed breast milk
EEG..... electroencephalography
EFA..... essential fatty acid
EP..... evoked potential
ERG ..... electroretinography
G_a/G ..... (acoustic)conductance
GA..... gestational age
G_{diff}..... gradient difference
G_{dP}..... gradient pressure difference
G_{ratio}..... gradient ratio
H_2O ..... water
IPL ..... interpeak latency
LA..... linoleic acid
L-A..... latency-age (function)
LBWI ..... low birth weight infant
LCP ..... long chain polyunsaturate
LCPUFA..... long chain polyunsaturated fatty acid
L-I ..... latency-intensity (function)
L-R..... latency-rate (function)
MEE ..... middle ear effusion
MLR..... middle latency response
NICU..... neonatal intensive care unit
OM..... otitis media
OME..... otitis media with effusion
PAS ..... peripheral auditory system
PC ..... personal computer
PCA..... post-conceptional age
PCV..... packed cell volume
PUFA ..... polyunsaturated fatty acid
R_a/R..... (acoustic) resistance
RBC..... red blood cell
SD ..... standard deviation

SN10 ..... slow negative wave
SOM..... serious otitis media
SN ..... sensorineural
SPL..... sound pressure level
SVP ..... slow vertex potential
TM ..... tympanic membrane
VEP..... visual evoked potential
X_a/X ..... (acoustic) reactance
Y_a/Y ..... (acoustic) admittance
Z_a/Z ..... (acoustic) impedance

dB..... decibel (nHL)
cm..... centimeter
daPa..... decaPascal
s..... second
g ..... gram
Hz..... hertz
kHz..... kilo hertz
kΩ ..... kilo ohm
pps..... pulses per second
μs ..... microsecond
μV ..... microvolt
mm ..... millimeter
ms..... millisecond

Introduction

Original Study Protocol

The reason for the original study was to investigate the relationship between diet and the maturation of the auditory system in the preterm infant. Arachidonic acid (AA) and Docosahexaenoic acid (DHA) had been identified as being of benefit to the maturational process of the immature auditory system of the preterm infant. It was stipulated that auditory testing, using the Auditory Brainstem Response (ABR) should be utilized to assess the neurological function during the preterm period. Infants born <32 weeks gestational age (GA) or <1500g were identified as being suitable for a study of this nature. This would obviously involve the testing of incubated infants in the Neonatal intensive care unit (NICU) environment.

The dietary investigation was to be undertaken using preterm formula feeds supplied by Milupa UK Limited. A test formula was produced that had been enriched with a long chain polyunsaturated fatty acid (LCPUFA) composition, another formula (devoid of LCPUFA enrichment) being used as a control. A further control group of breastfed (EBM) infants was also to be included. The study was to be performed blind. Blood chemistry analysis was to be performed in conjunction with the auditory assessment. At an early stage in this research the formula milks were changed due to the introduction of low level fatty acid compositions to standard preterm formulas. The test formula, used for this research, was thus altered to Milupa Prematil (enriched with a LCPUFA composition). The control formula, devoid of LCPUFA, was produced (for the study) by Cow & Gate Limited. The original study protocol, as proposed by Hillingdon Hospital, can be seen in Appendix A. This protocol sets out the recruitment and blood testing procedures that were to be used.

Protocol Development

The original study protocol identified the criteria for infant recruitment and the procedures for the blood chemistry analysis. An auditory testing protocol (using ABR) was not stipulated. It was, therefore, necessary to examine auditory testing procedures and protocols in order to achieve comprehensive results data. A detailed review of protocols, procedures and obtainable results was performed.

Dietary grouping criteria (>75% volume of a particular feeding regime) was observed. However, the defined group sizes (n=50) were found to be unrealistic for the time frame of this thesis. The number of infants with the birth criteria stipulated was found to be low. The recruitment of infants, at the time of birth, was also found to be impractical. Mothers were understandably not agreeable to enrolment at that time. This made collection of cord blood impossible. It was decided that recruitment of infants within the first week after birth was more appropriate.

A term study was implemented as stipulated in the original protocol. Term infants were recruited from mothers with no family history of auditory impairment and where the infants were considered as having a low risk birth with no complications. Mothers undergoing caesarean section were targeted due to their increased stay in hospital (approximately 5 to 6 days after delivery). This allowed for testing to be performed, with the mothers' consent, before discharge. These infants were tested with the same protocol as the preterm infants. Infants were not tested within the first days after birth to allow for ME normalization.

Staff of the newborn Hearing Unit were notified of deliveries of babies <32 weeks GA or <1500g. Parents were then approached by medical staff for inclusion on the study. Explanatory notes and consent forms were made available. Some problems were encountered with the organization of the recruitment in the NICU.

Mothers' blood, and that of the infant, were taken soon after recruitment. Blood samples from the infant were then taken in conjunction with auditory testing. The majority of subjects were tested until discharge from the hospital. Recalling infants after discharge was found not to be feasible. A number of subjects discontinued the study due to medical complications or transfers to other hospitals.

Mothers were advised by medical staff of a suitable feeding strategy for their babies. Those opting for formula feed were assigned one of the two study formulas. However, it was often found that those mothers that selected breast feeding did not produce sufficient milk supplies. These infants were then switched to, or supplemented with, one of the preterm formulas. Infants remained on preterm formula until advised by medical staff, this usually coincided with discharge from the hospital. A record of all feeding (including supplements) was kept by intensive care staff. The fixed testing days stipulated by the original protocol were found to be inappropriate. Test sessions had to be arranged to suit the intensive care staff, the babies feeding regime and parental visits. Testing was performed with a member of staff from the Newborn Hearing Unit who also had intensive care experience.

The original study protocol did not define an actual test protocol for the auditory assessment. A detailed literature review was performed in order to identify a test protocol suitable for both term and preterm infants. Initial testing was also performed on term and preterm infants to establish valid test procedures. This was especially important for incubated infants in the NICU environment. ABR testing was performed using a Bio-Logic Navigator Evoked Potential machine located in the Newborn Hearing Unit. It was proposed that term infants, and preterm infants not requiring intensive care facilities, would be tested within the Newborn Hearing Unit. The Bio-Logic Navigator would be taken to the NICU for testing of incubated infants and those receiving medical attention. Initial testing with this machine allowed for optimal settings to be identified for testing in the NICU environment.

Additional Auditory Assessment

To supplement the defined protocol, tympanometry was identified as an additional area of research. This procedure allows for assessment of middle ear (ME) function. It was proposed that this additional testing would provide a more complete assessment of the entire auditory system. This testing is also useful in identifying possible modifications to the electrophysiological data (obtained by ABR) due to ME function. A Grason-Stadler GSI33 Middle Ear Analyser was purchased for this work. This instrument was capable of collecting detailed tympanometric data. A standard screening

tympanometer was found to give insufficient information of ME function in the neonate population. The capability of the GSI33 instrument allows a detailed study of the mechanics of the ME system in the term and preterm infant. The utilization of this level of testing with these populations (especially preterm) is still limited. Tympanometry will be performed in conjunction with the ABR testing. The tympanometric testing was implemented later in the study, therefore, it was not possible to acquire tympanometric assessment from all preterm infants. This assessment was, however, performed on all infants recruited onto the term study.

Test session time limits were found to be an important factor in the identification of suitable test protocols. This was particularly important with the introduction of the tympanometric testing. Test sessions were arranged, where possible, not to coincide with parental visiting time and medical treatment. A limit of approximately 1½ hours was found to be appropriate. Standard ABR screening was also undertaken during the research testing to avoid the need for additional testing of the infants. This data was passed to the Paediatric Audiology Department for further assessment.

Thesis Structure

Four main areas were identified for the literature review:

1. Current opinion on the physiology of the auditory system to establish an appropriate physiological model for discussion of the results.
2. Auditory brainstem response (ABR) for implementation of appropriate test procedures and protocols.
3. Tympanometry to establish test procedures and a test protocol in order to achieve useful and informative data.
4. Infant nutrition to provide depth to the dietary reasoning behind the overall study.

The chapter contents are as follows:

Chapter One - The general anatomy of the overall auditory system is presented from middle through to inner ear, and beyond into the central auditory pathways. The physiological aspects of hearing are examined with particular emphasis on neural transmission and synaptic function. The current opinion on maturational aspects of the auditory system during the preterm period are investigated with reference to tympanometry and the ABR. The maturational characteristics of the ABR are reviewed and a physiological model established.

Chapter Two - Auditory brainstem response (ABR) collection parameters are examined to ensure successful, repeatable and reliable testing. Testing within the Neonatal intensive care unit (NICU) environment is assessed. A suitable test protocol is defined to allow the maximum amount of useful information to be obtained within the test time available. The detectability of the ABR from preterm infants in the NICU environment is investigated. Validation of the test protocol and procedures by initial testing of term and preterm infants is presented.

Chapter Three - The general principles and mathematical concepts of tympanometry are presented. The implementation of multi-frequency, multi-component tympanometry in

the term and preterm infant is discussed. A test protocol is established to produce a safe and reliable procedure for testing infants in the NICU. A broad test protocol is developed in order to obtain the maximum amount of informative data. Various tympanogram descriptors are also investigated to provide useful quantifiers for numerical data interpretation.

Chapter Four - The interaction of arachidonic (AA) and docosahexaenoic (DHA) acids with neurological development is reviewed. The role of infant nutrition in early development is considered. The methods of providing the preterm infant with sufficient levels of AA and DHA is discussed. The review examines the inclusion of linoleic (LA) and α-linolenic (ALA) acids, the precursors to AA and DHA, into preterm formula feeds.

Chapter Five - The ABR results for both preterm and term infants are presented. Maturational characteristics for the preterm population are investigated. The effects of the preterm birth are examined by comparison of term and preterm data during the term period. The established physiological model is utilized to investigate the maturation time course of different regions of the auditory system and to understand the differences between intra- and extra-uterine maturational characteristics. An analysis of possible gender and dietary effects is undertaken.

Chapter Six - The results of the tympanometry study are presented. Both term and preterm infant data are discussed. The Vanhuyse classification system is assessed and the morphology of susceptance and conductance tympanograms at various frequencies reported. The usefulness of tympanometric descriptors are investigated with particular reference to the reliability of use with the neonate population. Infant middle ear function and the effect of the neonate external auditory meatus on tympanometric data is discussed. The effect of prematurity on the characteristics of tympanometry are assessed and maturational trends for the preterm infant identified.

Chapter Seven - Appropriate statistical and analytical methods are established. The maturational characteristics of the preterm ABR are examined for the various regions of the auditory system. The effect of the preterm birth is investigated with a comparison of mean data collected during the term period from both term and preterm infants. Possible gender and dietary effects are analyzed. Normative characteristics of term and preterm multi-component tympanometric data is presented. The maturation of preterm tympanometric data is examined and the validity of this, and term data assessed. References are made to the clinical application of multi-component tympanometry with the neonate population. A comparison of ABR and tympanometric data is undertaken to establish the different conductive characteristics of term and preterm peripheral auditory system.

Chapter Eight - The conclusions of the study for both ABR and tympanometric testing are presented.

Chapter Nine - Areas of further work for calloboration of the current study are identified. Related areas of work for future research are highlighted.

Appendices are presented for the original study protocol (as proposed by Hillingdon Hospital) and the mathematical concepts of tympanometric testing. This information can be found in Appendices A and B respectively. Appendices C and D show the raw data for the term and preterm ABR testing respectively.

Additional information is presented in Appendices E and F. Acoustic reflex measurements were made during tympanometric testing. However, the testing that was most appropriate to this thesis was not possible due to procedural difficulties. The results that were collected are presented for reference in Appendix E. Some work was also carried out into a finite element model of the infant tympanic membrane. The program which was developed during this work is presented in Appendix F. Time was not sufficient for this area of research to be examined in depth. Both reflex measurement and the finite element model will not be discussed in this thesis.

Study Aims and Objectives

These aims and objectives have been formulated in order to provide focus and direction for this study. The following areas of research will be investigated.

Auditory Brainstem Response (ABR)

- To assess descriptors of auditory maturity, including various parameters and functions relating to maturity indices.
- To investigate the maturational time course of the various regions of the auditory system in the preterm infant.
- To develop the understanding and usefulness of ABR synaptic efficacy indices.
- To assess the effects of the preterm birth by the term period for both the peripheral and central auditory systems.
- To examine possible gender effects on both the term and preterm neonate populations.
- To investigate the interaction between long chain polyunsaturated fatty acid (LCPUFA) enriched formula and ABR parameters.
- To investigate the link between peripheral auditory system ABR data and tympanometric measures.

Tympanometry

- To produce normative data characteristics for multi-frequency, multi-component tympanometry in term infants.
- To establish useful descriptive techniques for tympanometric data from both term and preterm infants.
- To investigate the complex characteristics of tympanometric data during the preterm period.
- To examine the effect of preterm birth on middle ear function in the neonate.
- To assess the maturational consequences, to tympanometric data, arising from a preterm birth. A comparison of data from preterm (during the term period) and term born infants will be undertaken.
- To validate the mathematical concepts underlying the use of tympanometry in the neonatal population.
- To assess the reliability of infant data.
- To develop the clinical application of multi-component, multi-frequency tympanometry in the neonatal population.

CHAPTER ONE

THE AUDITORY SYSTEM AND ITS MATURATION

1.0 Specific Deliverables

- Review of the anatomical aspects of the middle and inner ears, and the central auditory pathways.
- Review of current opinion of physiological aspects of hearing. In particular, the transmission properties of the central auditory pathways.
- Investigation of the maturational aspects of the middle and inner ears, and the central auditory pathways.
- To establish the neural generators of the ABR.
- To establish a physiological model for ABR parameters from the current literature.
- Review of infant ABR characteristics and maturational aspects of the ABR.

1.1 External and Middle Ears

The physical layout of the auditory system can be divided into outer, middle and inner ears; the auditory nerve; and the central auditory pathways. Figure 1.1 shows a cross-sectional view of the outer, middle and inner (cochlea) ears. The external ear consists of the auricle or pinna and the external auditory meatus (EAM) or ear canal. The outer portion (approximately one-third of its length) is cartilagenous, with the remaining two-thirds being bony. The canal is lined with tight-fitting skin that is thicker in the outer cartilagenous section. The canal is not straight and takes a slightly irregular course leading to the tympanic membrane (TM) or eardrum. The external ear collects sound waves which cause the TM to vibrate. The TM sits at an angle of approximately 55° to the plane normal of the ear canal as shown in Figure 1.1. The membrane is thin and translucent. The TM is concave when viewed from the ear canal. The peak of the cone is

the umbo. This inward displacement is associated with the attachment of the TM to the manubrium of the malleus.

The vibratory motion is transmitted through the middle ear (ME) system. The ME cavity or tympanum contains a chain of ossicles; namely, the malleus, incus and stapes. The tympanum also contains the opening of the Eustachian or auditory tube. This tube helps enable an equal air pressure on either side of the TM. It also allows for drainage of the ME by serving as a portal into the nasopharynx. The first third of the tube leaving the tympanum is surrounded by bone with the remainder being enclosed within an incomplete ring of hook shaped elastic cartilage. The cartilaginous part of the Eustachian tube is normally closed. It opens reflexly by the action of the tensor palatini muscle. This muscle uncurls the normally hook shaped cartilages in response to swallowing, yawning, sneezing or shouting.

The three bones of the ME are collectively termed the ossicular chain (fig. 1.2). The ossicular chain is suspended in the ME by a series of ligaments, by the tendons of the two intratympanic muscles, and by the attachments of the malleus to the TM and of the stapes to the oval window. The malleus (commonly called the hammer) is attached by its manubrium to the mucous membrane and fibrous layers of the TM. It is also suspended by its superior ligament. A projection just below the head of the malleus connects the malleus with its anterior ligament (fig. 1.2), this forms the axis of malleur movement. There is a further connection at the top of the manubrium via a tendon to the top of the tensor tympani muscle.

The incus (referred to as the anvil) has its body connected to the head of the malleus at the malleoincudal joint as shown in Figure 1.2. It is now known that the nature of this connection results in the malleus and incus moving as a single unit[1] rather than relatively as had been previously thought. The short process of the incus is connected to the posterior wall of the tympanic cavity by its posterior ligament. Its long process runs parallel to the manubrium then bends to articulate with the head of the stapes in a ball and socket joint. The stapes (or stirrup) is the smallest ossicle. The head of the stapes connects to the footplate via two crura. The footplate is then connected to the oval window by the annular ligament. The footplate encases the very fine stapedial membrane. The stapedius tendon is attached to the neck of the stapes and connects the bone to the stapedius muscle. The faceplate of the stapes lies against the membranous

labyrinth in the opening of the oval window. The process transmits the sound energy to the inner ear, the cochlea.

1.2 Inner Ear (Cochlea)

The inner ear structures are contained within a system of spaces and canals called the osseous or bony labyrinth. It is located in the petrous[a] portion of the temporal bone. This labyrinth can be grossly divided into three sections; the vestibule, the cochlea, and the semicircular canals. The oval window accepts the footplate of the stapes and opens into the vestibule.

The spaces between the bony walls of the osseous labyrinth and the membranous labyrinth are filled with a fluid called perilymph. The membranous labyrinth is itself filled with endolymph. The hair cells of the cochlea are based in a third fluid called cortilymph. The functions of these fluids was described by Lawrence[2], he stipulated four basic functions.

1. They deliver nutrients to the inner ear cells which are not in direct contact with the blood.
2. They provide the physical medium for the transfer of energy from vibratory stimuli into a neural signal.
3. They aid transfer of vibratory stimuli from the footplate to the sensory structures along the cochlear partition.
4. It has been debated that the pressure in the inner ear is controlled by the cochlear fluids.

These fluids have similar properties to other fluids involved in neuronal processes further into the auditory system. Perilymph is similar to other extra-cellular fluids; the principal cation being sodium. It has a very high concentration of sodium and a very low concentration of potassium. The reverse applies to the endolymph, where the principal cation is potassium, with a low sodium concentration[2]. Perilymph and cortilymph have a similar composition to extra-cellular fluid; sodium being the principal cation. Endolymph

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[a] Thicker and denser part of the temporal bone protecting the middle ear.

has a composition closer to intra-cellular fluid; the principal cation being potassium. Because of the relative cation concentrations, perilymph and cortilymph assist the function of hair cells, while endolymph tends to inhibit.

The cochlea forms a cone-shaped spiral, it is widest at the base and tapers towards the apex (fig. 1.3). At the axis of the cochlea spiral is a core (modiolus) through which the auditory nerve and the blood vessels that supply the cochlea pass. A bony shelf called the osseous spiral lamina spirals around this core. The basilar membrane is attached to the osseous spiral lamina and proceeds with it up the cochlea (fig. 1.3). The structure of the cochlea can be seen in Figure 1.4 which shows a cross-sectional view. It displays the cochlear canal making two and a half turns around the modiolus. The modiolus contains channels for blood vessels and branches of the cochlear nerve. The top two turns in Figure 1.4 show the scala vestibuli, cochlear duct and the scala tympani which compose the cochlear canal (right) with the membranes and the organ or Corti shown on the left side. The five spiral views make up the two and a half turns of the spiral.

The configuration of the cochlea is clearer when considered in an unspiralled state. Figure 1.5 shows the three chambers; the scala media (or cochlear duct), scala vestibule, and the scala tympani. These cavities compose the cochlear canal. The scala media (containing endolymph) is self-contained and separates the other two. The scalae vestibuli and tympani can communicate with one another at the apex of the cochlea. The membranous labyrinth encloses the central scala media. The scala vestibuli is in contact with the stapes at the oval window (fenestra vestibuli) whilst the scala tympani has a membrane-covered contact with the ME at the round window (fenestra cochleae). The scala media (or cochlear duct) is separated from the scala vestibuli above by Reissner's (vestibular) membrane and from the scala tympani below by the basilar membrane. A cross-section of the cochlear duct is shown in Figure 1.6.

Bekesy[3] reported that the basilar membrane tapers from the apex to the base near the stapes. In addition, that it progressively thickens nearer the narrower base end. The basilar membrane is of great importance to the physiology of hearing. It responds to vibration of the stapes. This vibration produces waves in the perilymph of the vestibule. Sound waves propagate through the scala vestibuli, Reissner's (vesibular) membrane, the endolymph in the cochlear duct, and the basilar membrane to the scala tympani. These

waves then create a vibration of the membrane closing the fenestra cochleae at the base of the scala tympani. This eliminates the damping of the pressure waves that would otherwise occur in a bone-encased fluid. The helicotrema connects the two perilymph compartments at the apex of the cochlea (shown in fig. 1.5) but serves only to equalize the fluid pressures. Sitting on the basilar membrane is the end organ of hearing, the organ of Corti.

The organ of Corti runs longitudinally along the basilar membrane. It is located between the pillar cells of Figure 1.6. It consists of a single row of inner hair cells (IHC), three (up to 4 or 5 in some locations) rows of outer hair cells (OHC) and various supporting cells. Each of the IHCs (approximately 3,500) is supported by a phalangeal cell that holds the rounded base of the hair cell. The 12,000 OHCs are supported by Deiters' cells (outer phalangeal cells). Lying between the inner and outer hair cells are the pillars (or rods) of Corti. These tilted pillars come together at their tops to enclose the tunnel of Corti (as shown in fig. 1.6). Auditory nerve fibres traverse this tunnel to contact with the OHCs. The cochlea produces action potentials which run through the central pathways of the auditory system.

1.3 Central Auditory Pathways

The cochlear (or auditory) nerve consists principally of axons (or central processes) of cells in the spiral ganglion. It transverses the internal acoustic meatus in the petrous part of the temporal bone alongside the vestibular nerve. Beyond the meatus, it continues to the junction of the medulla and pons. The fibres of the auditory nerve constitute the first-order neurons of the ascending auditory pathways. This is shown in Figure 1.7 where the dots show the start of neurons. These afferent fibres then bifurcate, one branch in the dorsal (DCN), and one in the ventral cochlear nucleus (VCN). The cochlear nuclei are located in the rostral end of the medulla.

The pathway to the cerebral cortex consists of variable numbers of synaptic relays between the cochlear nuclei and the specific thalamic nucleus for hearing, the medial geniculate body (fig. 1.7). The first-order neurons of the auditory nerve upon entering the brainstem synapse with cell bodies in the DCN and VCN as previously stated. Neurons arising from the more basal areas of the cochlea terminate in the DCN

and the VCN. The VCN also receives neurons originating in the more apical parts of the cochlea.

The brainstem pathways to the cerebral cortex are characterized by variable numbers of synaptic relays between the cochlea nuclei and the medial geniculate body (the specific thalamic nucleus for hearing). There is a relay in the inferior colliculus, with possible relays in the superior olivary complex and the nucleus of the lateral lemniscus. As can be seen in Figure 1.7, there are significant ipsilateral and contralateral projections to the cortex.

Some fibres from the VCN proceed to the region of the ipsilateral superior olivary nucleus. However, the majority of fibres from the VCN decussate the pons to form the trapezoid body as shown in Figure 1.7. At the superior olivary nucleus region some fibres continue into the lateral lemniscus with other terminating in the superior olivary nucleus. Fibres are added from the superior olivary nucleus to the lateral lemniscus. Fibres from the DCN decussate and continue obliquely to the contralateral superior olivary nucleus. Fibres then continue in the lateral lemniscus and end in the inferior colliculus.

Signals conveyed by the lateral lemniscus reach the inferior colliculus in the midbrain (with or without a synaptic relay in the nucleus of the lateral lemniscus). This possible relay is not shown in Figure 1.7. The neuronal organization is complex in the inferior colliculus. This indicates complex integrative activity at this level. Fibres from the inferior colliculus end in the medial geniculate body. Signals are then projected to the primary auditory cortex. It should be noted that there also exists descending fibres (efferent fibres) in the auditory pathway that conduct in the reverse direction.

1.4 Central Pathway Structure

The cells of the central nervous system (CNS) are more varied than those in any other part of the body. The neuron consists of the nucleus and the cells communicating processes, the dendrites and the axon, originate from the cell body. Neurons are one of the excitatory cells of the body. They undergo rapid shifts in transmembrane potential because of the flow of ions into and out of the cells.

1.4.1 Membrane Potentials and Action Potentials

A fibre (or axon) can be considered as a tube filled with a watery solution of salt (dissociated into positively and negatively charged ions) and proteins. These are separated from the extra-cellular fluid by a semi-permeable membrane. The solutions are of the same ionic strength but have different ionic composition. Ions can move through specific channels that span the membrane. Stimulation causes the various channels to open and close. The electrical potential difference across the nerve cell membrane is dependant on the ionic concentration gradient across it and the relative permeability of the membrane to the ions present. For a steady-state to be maintained, the total distributions on either side of the membrane must meet three constraints.

1. The bulk solutions inside and outside of the cell must be electrically neural.
2. The osmotic concentration of the intra-cellular ions and molecules in solution must equal that in the extra-cellular fluid.
3. There must be no net flux of any permeant ion across the membrane.

The major components contained by the cell are sodium, potassium and chloride which can diffuse across the cell membrane; and protein which cannot diffuse. The sodium, potassium and chloride diffuse at different rates. Each of the permeant ion species has different intra- and extra-cellular concentrations. There are two gradients to drive ions in and out of the cell; a concentration gradient and an electrical gradient. Potassium is more concentrated inside the cell than out and thus outward movement along the concentration gradient is expected. However, the inner surface of the membrane is negative with respect to the outside which tends to restrain the outward movement of the positively charged ions. In resting cells the concentration and electrical gradients are almost balanced. This process of electrical and osmotic differences across the membrane in the resting state is known as Donna equilibrium. Sodium and chloride have greater concentrations outside of the cell. They behave with similar but reversed characteristics as the potassium. The resting potential depends on the relative permeabilities of the cell membrane to sodium and potassium. As part of this steady-state

condition there is also an active transport process (active pump) for the sodium and potassium. Proteins transport sodium ions out of the cell and potassium ions in.

Generation of nerve impulses occurs when depolarization increases sodium permeability and, more slowly, potassium permeability. It is the voltage dependence of sodium and potassium permeabilities that is responsible for the action potential. The action potential will also cause adjacent portions of the membrane to be excited. This results in propagation of the action potential. The electrical charges of the membrane potential will affect the core of the axon for several millimetres. In large fibres, this behaviour will increase voltage for 1-3 mm, thus initiating further action potentials. This process (nerve impulse) will propagate throughout the fibre. This is the method of nerve impulse transmission in unmyelinated fibres.

1.4.2 Nerve Fibre Myelination

The central core of the nerve fibre is the axon, its membrane is the actual conductive element. The centre of the axon is filled with axoplasm, an intra-cellular fluid. As current spreads along an axon, it becomes attenuated with distance. This attenuation is dependant principally on axonal diameter and membrane properties. Spread is greater for larger diameters and a higher membrane resistance. A myelin sheath has a high resistance making it an effective insulator for current to be conducted with speed between nodes. This sheath can be thicker than the axon itself. It is laid around the axon in multiple layers. These layers decrease the ion flow through the membrane by approximately 5000 times[4]. Interruptions of the myelin sheath at 1-3 mm intervals, called Nodes of Ranvier, occur along the length of the axon (fig. 1.8).

At the various nodes there exists a region (2-3μm in length) of uninsulated axon where there is no myelin sheath. In these areas, the ions can flow with ease between the axon and the extra-cellular fluid. With the properties of the myelin sheath, action potentials cannot be generated within the majority of the axon length. Therefore, the only locations where action potentials can be produced is in the immediate area of a node. The process of conduction from node to node in myelinated fibres is term 'Saltatory Conduction'. The electrical current flows from node to node through the axoplasm of the axon and through the surrounding extra-cellular fluid.

This method of conduction has two valuable characteristics. Firstly, the velocity of nerve transmission is greatly increased by allowing the depolarization process to jump over long distances along the axon from node to node. This can increase transmission velocity by approximately 5 to 50 fold[4]. Secondly, axon energy is saved due to the depolarization process only occurring at node sites. This eliminates the need for the depolarization process along the complete length of the axon. It is possible that this leads to a hundred times smaller number of ions lost than would otherwise be required for the same nerve impulse. This reduces the requirement for re-establishment of the sodium and potassium concentration differences across the membrane after a series of nerve impulses. The velocity of conduction in nerve fibres varies considerably from 0.5m/s in very small unmyelinated fibres to 100m/s in large myelinated fibres.

There are also periods when excitation in a nerve fibre cannot happen, this is during the refractory period. New action potentials cannot be elicited as long as the membrane is still depolarized from the preceding action potential. Another action potential can only be produced when the membrane potential has returned to, or almost to, the resting potential. The refractory period for a large myelinated fibre is approximately 0.4ms[4]. This provides for a maximum rate of 2500 impulses per second.

1.4.3 Organization of the Central Nervous System (CNS)

The process by which nerve impulses are transmitted along the axon body in the neuron have been discussed. The incoming information for a cell enters through synapses in the neuronal dendrites or cell body. There are between 200-200,000 synaptic connection from the input fibres. The output signal travels along a single axon. This axon then separates many times to provide synapses with the next order of neurons.

All synapses in the CNS are chemically based. The first neuron (presynaptic neuron) will secrete a chemical substance called a neurotransmitter at the synapse which act on receptor proteins in the membrane of the next neuron (postsynaptic neuron). Considering first the presynaptic neuron, the synaptic membrane contains large numbers of calcium channels. When an action potential depolarizes the synaptic terminal the calcium ions (with sodium ions that predominantly cause the action potential) flow into the terminal. The number of these ions directly influences the quantity of transmitter

substance that is released into the synaptic cleft. The postsynaptic neuron contains large numbers of receptor proteins at the synapse. These receptors consist of a binding component that protrudes outwards into the synaptic cleft and an ionophore component that passes inwards through the membrane into the postsynaptic neuron. It should be noted that receptors can cause both excitation and inhibition. This allows for restraint of neuron action as well as excitation. The resting membrane potential is less negative than that found in the nerve fibre. It is this characteristic that allows for the excitatory and inhibitory control of the synapse. A less negative value will excite whilst a more negative potential will inhibit.

Any synapse will have a delay in transmission associated with it, the action potential will be delayed when passing from the pre- to post-synaptic terminals. The observed delay is caused by a number of processes, these include the time taken for the pre-synaptic terminal to discharge the transmitter substance and the subsequent diffusion of the transmitter through the post-synaptic neuronal membrane. The transmitter then has to act on the membrane receptor and increase the membrane permeability. There then follows an inward diffusion of sodium to raise the excitory post-synaptic potential to a value high enough to elicit an action potential. The transmission time for these processes is generally around 0.5ms for the adult human.

In addition to this basic synaptic delay, a process known as synaptic fatigue also exists. This addition delay is evident when excitory synapses are repetitively stimulated with a rapid rate stimulus. The number of discharges at the post-synaptic neuron is initially great, but becomes progressively less in the following milliseconds or seconds. The amount of reduction can be dependant on the status or maturity of the auditory system. The function of his delay is also to reduce excessive excitability with time in the case of excessive neuronal activity in a protective manner. This fatigue behaviour results from an exhaustion in the supply of transmitter substances in the synaptic terminals. Most neurons store enough transmitter substance for 10,000 normal synaptic transmissions. Other causes of synaptic fatigue include the progressive inactivation of many of the post-synaptic membrane receptors and a slow build up of calcium ions inside the post-synaptic neural cell caused by successive action potentials. The build up of the calcium ions by the successive action potentials in turn opens calcium activated potassium channels causing inhibitory effects on the post-synaptic neuron.

1.5 Maturation of the Auditory System

1.5.1 Peripheral Auditory Structures

Studies have been performed[5, 6] on animals have shown that direct stimulation of the auditory nerve can elicit an evoked response from the auditory cortex soon after birth when behavioural responses to sound are not observed. It is concluded that this is evidence that the auditory pathways in the brain are mature enough to respond to sound. Thus, the limiting factor preventing responses to auditory stimuli are the immaturities peripheral to the auditory nerve.

To further differentiate between possible ME and inner ear immaturity Geal-Dor *et al.*[6] reported ABR thresholds to air-conducted and bone-conducted stimuli in the rat. The bone-conduction method applies vibrations to the skull stimulating the inner ear directly. Research on altricious species allows for investigation of the onset of auditory function in the extra-uterine environment after birth. This behaviour can be correlated to the onset of auditory function in the human foetus in utero. They reported that whilst the ABR could be recorded at post-natal day (PND) 7 for bone-conduction, the air-conducted stimuli did not elicit an ABR until PND 11. Geal-Dor *et al.*[6] concluded that the inner ear matures earlier than the ME. Thomas and Walsh[7] reported that the ME ossicle impedance matching mechanism was not a significant limiting factor in the cat at PND 7. They found the TM/stapes footplate ratio was adult-like at this age. Sohmer and Freeman[8] concluded that the maturational processes occurring in the ME between PND 9 and 15 include resorption of the ME mesenchyme, ossicular ossification and opening of the ear canal.

Sohmer and Freeman[8] also reported the possible processes responsible for reducing ABR thresholds for the inner ear. Bosher and Warren[9] suggested that the maturation process is due to an increase in the resistance of the membranes surrounding the scala media. However, Sohmer and Freeman[8] suggested that a more probable cause is related to the increase in sodium and potassium activity. This increase would lead to a larger endocochlear potential of the scala media. This larger endocochlear potential would, they argue, lead to improved thresholds by providing a larger potential difference across the apical surface (stereocilia) of the IHCs resulting in enhanced transduction currents. Sohmer and Freeman[8] suggested that the larger endocochlear potential would

also lead to improved auditory sensitivity by increasing the activity of the cochlear amplifier which is related to OHC function. They argue that this would result in enhanced displacement of the basilar membrane. Oto-acoustic emissions (OAE) can be recorded in the external auditory canal to assess the micromechanical displacement of the basilar membrane induced by the electromotility of the OHCs.

The characteristics of the onset of auditory function in altricious species are useful in the investigation of human auditory function. The human foetus is precocious and is able to hear from birth[10]. The in utero onset of auditory function is confirmed by the ability to produce changes in foetal heart rate by vibro-acoustic stimulation delivered to the maternal abdomen[11]. Of course, this result is further supported by the elicitation of ABR in preterm infants as early as 26 weeks GA[12]. In addition, the ability to detect OAEs in preterm infants confirms that the conductive (external and ME) and sensorineural (inner ear) mechanisms are at a fairly advanced state of maturation.

Sohmer and Freeman[8] suggested that the sequence of maturation leading to the onset of auditory function is similar in precocious and altricious species except that the rate of change is different. They link the onset of auditory function with thyroid hormone levels and suggest that a surge is observed in the altricious species at the onset of auditory function. It should be noted that thyroid hormone surges could be a marker for other events. The surge in thyroid hormone levels in the human occurs at 21 weeks GA, reaching half maximum levels by 26 weeks GA[13]. This surge leads to a series of conductive (external and ME) and sensorineural (inner ear) maturational events, the details of which are still not clearly defined. Sohmer and Freeman[8] reported that these events include development of the organ of Corti, ME mesenchyme resorption, hardening of the ossicles, increased concentrations of sodium and potassium, development of the endocochlear potential and improvement of the auditory threshold. Pujol *et al.*[14] reported that the structural development of the human cochlea is mature by 30 weeks GA. Eggermont *et al.*[15] suggested mature cochlea function by 35 weeks GA.

OAEs have been used to assess cochlea function in neonates. An OAE is defined as any sound that originates from the cochlea and can be recorded in the outer ear canal[16]. Ochi *et al.*[17] and McNellis and Klein[18] commented on the effectiveness of OAEs with the preterm population. They concluded that OAEs presented comparable results to those obtained by the more complex examination of the ABR. Chuang *et al.*[19] recorded

OAEs in preterm infants and found that the characteristics of emissions change throughout the preterm period. They correlated the increasing cochlea performance with latency changes observed in the ABR. This result would imply that a maturation of the cochlear transduction component is present within the preterm period. Chuang *et al.*[19] note that the characteristics observed with their results could be influenced by alterations in ME properties. However, they suggest that changes in ME characteristics at this age would not be age-dependant as seen with maturational behaviour but due to transient ME conditions.

1.5.2 Neural Development

The main events concerning neural foetal development are proliferation of the total complement of neurons, migration of those neurons to specific sites throughout the CNS, a series of organizational events that result in the intricate circuitry characteristics, and ensheathment of this circuitry by a neural-specific lipid compound called myelin[20]. These processes span from the second month of gestation into adult life. Kawarai *et al.*[21] reported that developmental changes in the CNS are strongly linked to audiological evaluation utilizing ABR during the preterm period.

Neuronal proliferation occurs initially between the second and fourth months of gestation. The migration of nerve cells from their sites of origin to loci within the CNS occurs between approximately the third and fifth months of gestation. The organizational events, occurring from the fifth month onwards, are numerous and complex. They include, amongst others, alignment, orientation and layering of neurons, elaboration of dendritic and axonal ramifications, and establishment of synaptic connections. These events are of particular importance because they establish the elaborate circuitry of the CNS and they set the stage for the final development event, namely myelination.

Myelination starts in the second trimester of pregnancy and continues into adult life. This process starts with a rapid proliferation of glia, some of which differentiates to oligodendroglia and align along axons. The plasma membranes of the oligodendroglia become elaborated as the myelin membrane of the CNS. The myelination processes occur at different rates and at different periods during development. Much development occurs after birth (post 40 weeks PCA). Yakovlev and Lecours[22] produced a description

of myelin developmental times for 25 areas of the human CNS. From their work it can be stated that myelination first occurs in the peripheral nervous system. The sensory roots in the peripheral nervous system are the second area to start myelination (6 months of gestation), just after the motor roots. Shortly after this (<7 months), myelin appears in components of major sensory systems. The auditory components include the lateral lemniscus, the trapezoid body and the brachium of the inferior colliculus. Unlike the peripheral nervous system, myelination in the CNS occurs first in sensory rather than motor systems. Higher level structures will tend to start myelination around 40 weeks gestation and continue throughout the first year of life and beyond into adulthood.

It is established that the human brain is incomplete at birth for both term and preterm infants. The developmental myelination process is in its early stages and will continue further. Whilst the post term stages of myelination have been studied in depth[23, 24, 25, 26] there is still a lack of knowledge of the preterm stages of development. Magnetic Resonance Imaging (MRI) allows for the visualization of white matter development in vivo. Van de Bor *et al.*[27] used MRI to compare myelin status in the CNS between term and preterm infants. They performed MRI at 44 weeks PCA on 10 term and 14 very preterm infants (<32 weeks GA). They concluded that, whilst the preterm infants generally had lower body weights, there was no apparent qualitative differences in CNS myelination between the two groups. All infants had reached the qualitative myelination stages previously reported for term infants at 44 weeks PCA.

Huppi *et al.*[28] studied the effects of the preterm birth and extra-uterine environment on the prematurely born infant. Myelination was assessed in vivo using MRI. They compared the maturation of the preterm infants exposed to both intra- and extra-uterine environments with the solely intra-uterine environment of the term infant. The preterm infants were tested after 2 weeks of life and at term PCA. They found that there was an increase in myelination between 32 and 40 weeks PCA in the preterm infants in the extra-uterine environment. They reported that the rate of change was similar to that observed from autopsy studies of foetuses of various GA. They also studied the effect of the extra-uterine environment by comparing the later preterm data with a control term group. They found the myelination was retarded in the preterm infants in comparison to their term counterparts. They concluded that the preterm infant grown to term will be uniformly less mature in relation to myelination status.

Synaptic development occurs during the period of organization within the CNS. Initially, dendrites appear as thick processes with only a few fine spicules. As development progresses, a greater number and variety of dendritic spines appear, the sites of synaptic contacts. The basic principles of development are the formation of early synapses, a peak to excess of the adult number, then a subsequent period of synaptic elimination. Synaptic formation development is initially stimulated by activity-independent events such as molecular mechanisms involved in targeting. Activity-dependant events occur after the development of receptors on target neurons and the generation of electrical activity.

It is known that neural tissue (especially in the case of the cerebral cortex) possesses the propensity to change its function. This phenomeon is known as plasticity. In the cerebral cortex, when a region is destroyed, other regions can take over some of its function. This is particularly pronounced in young infants. It is probable that processes concerned with plasticity occur during the preterm period. It should be noted that reference to maturation of synaptic function can refer to the continuing development of existing synaptic connections and establishment or re-organization of new synaptic connections.

1.5.3 Nutrition in Neural Development

Nutrition plays an important role in the development of all newborn infants. However, this role is particularly vital for the prematurely born infant. The preterm extra-uterine period is a time of rapid development and is pivotal to continuing neurological integrity. The predominant process in neurological development for the preterm infant is the process of myelination. Myelin formation in the CNS occurs as a result of the expression of the genetic programme of the oligodendrocyte, the cell responsible for myelin synthesis[29]. As previously mentioned, the oligodendrocytes form large quantities of specialized lipid compound that wraps around axons and are then compacted into myelin lamellae. The oligodendrocytes develop from immature neuroectodermal cells deep within the CNS[29].

Studies[30, 31] have reported that among environmental factors that affect myelin development that nutrition plays an important role. The role of dietary lipids is evident

with lipid deposition and metabolism being intimately connected with biogenesis of myelin[32]. Essential fatty acid (EFA) deficiency is known to have an affect on myelin development[33, 34]. Normal cell function is dependant on two separate families of EFAs, the ω6 and ω3 series. The accepted acids essential for human development are linoleic (LA) and α-linolenic (ALA) acids from the ω6 and ω3 families respectively.

Wauben *et al.*[35] investigated preterm infants up to 6 months corrected age observing nutritional management. Infants received breastmilk with different multi-nutrient fortifiers. They concluded that the various fortifiers tested that the breastmilk without a fortifier provided a satisfactory nutritional supply. Tudehope and Steer[36] reported that the optimal diet for the preterm infant is one that supports a growth rate approximating that of the term infant developing in the intra-uterine environment. However, they stipulated that this should not impose stress on the developing metabolism or excretory systems. They concluded that even though breastmilk does not meet the energy and nutrient requirements of the preterm infant, the assorted medical benefits make it the diet of preference.

Uauy and Mena[37] reviewed the benefits of dietary long-chain polyunsaturated fatty acids (LCPUFA) on early development. They stated that breastmilk is the best and only time-proven source of fat and EFAs in the infant diet. They concluded that whilst the majority of formula feeds contain concentrated sources of LCPUFAs or precursors, there is still a need for better definition of optimal amounts and compositions. Crawford *et al.*[38] reported that arachidonic (AA) and docosahexaneoic (DHA) acid levels in the preterm infant drop below the intra-uterine expectation with both present enteral and parental feeds. They indicated that current EFA content for preterm feeding is incorrectly formulated for optimal neurological growth. The role of these acids is discussed in detail in Chapter Four on Infant Nutrition. The interaction of the specific polyunsaturated fatty acids (PUFA) being assessed in this study are also investigated in Chapter Four.

1.6 Neural Generators of the Auditory Brainstem Response (ABR)

This current research implements auditory brainstem response (ABR) evaluation to assess the maturation of the auditory system in the preterm infant. The majority of information on the neural maturation of the auditory system come from studies of the

ABR. Chapter Two contains details of the ABR testing implemented for this current study. It is accepted that the far-field[b] potential waves recorded from the scalp represent activity of internal auditory pathways and structures. The wave components of the ABR are believed to reflect activation of successive auditory brainstem nuclei and pathways. Buchwald and Huang[39] studied cat ABRs, they concluded that wave I originates from the auditory nerve, wave II from the cochlea nuclei, wave III from the superior olivary complex and the wave IV/V complex from the lateral lemniscus (see fig. 1.5). Moller *et al.*[40] carried out a similar study on humans, they agreed with the findings of Buchwald and Huang[39], suggesting that waves I and II both result from electrical activity of the auditory nerve. Pettigrew *et al.*[41] proposed that the large negative wave (II_n) was also generated by neural activity in the cochlea and auditory nerve.

For the maximal interpretation of the ABR, an estimation of the anatomical origins of the various ABR components is beneficial. It is generally accepted that scalp potential electrodes record far-field potentials generated by fibre tracts and nuclei of the ascending auditory pathway. Animal studies have formed the basis of knowledge for the neural generators responsible for hearing. These will be discussed in the next section. This current research is concerned with the maturational characteristics of ABR waves and in particular the interpeak latency (IPL) parameters. The use of rate attenuation characteristics is also being investigated in this current research. It is, therefore, necessary to examine the current thinking of the neural generators of the ABR peaks, the particular representations of IPL parameters, and the physiological assumptions regarding rate attenuation.

Many studies[42, 43, 44] have correlated their work with the neonate ABR to myelination of the auditory system. A few of these authors[45, 46] have extended this correlation to equate rate attenuation with synaptic interaction. For this current research, it is necessary to build an informed and appropriate model of the auditory system as research stands currently to facilitate a physiological discussion of the acquired ABR data. This current research is concerned with anatomical structures responsible for the generation of waves I to V. The particular waves I, III and V are of interest due to the high levels of detectability during the preterm period. The waves II and IV tend to be less robust and are really more suited to studies during the post term period.

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[b] Far-field refers to the electrical activity of the auditory system as recorded from the scalp.

For the correlation and investigation of maturational characteristics it is necessary to understand the possible physiological processes involved with the various latency and IPL parameters. It is the physiological processes of the IPL parameters that is of greatest interest. Ponton *et al.*[47] attempted to correlate anatomical development and evoked potential data. They developed a model of the anatomical structures and physiological processes responsible for the ABR waves I to V. They assumed that waves I and II are generated peripherally in the auditory nerve, with wave III being generated by axons leaving the cochlear nucleus. Waves IV and V also being generated centrally in brain stem structures.

It is, therefore, concluded that the I-III IPL reflects activity between the cochlea and cochlear nucleus (ie. the auditory nerve). The III-V IPL then reflects activity in the lower brainstem between the cochlear nucleus and the lateral lemniscus. Whilst the peripheral activity is generated by a singular pathway (auditory nerve), the central processes are thought to be more complex with parallel pathways[48]. Coleman and Clerici[49] suggested, from animal studies, that approximately one third of axons leaving the cochlear nucleus traverse the trapezoid body and lateral lemniscus to connect with the contralateral inferior colliculus without interruption. The uninterrupted nature of this pathway indicates an asynaptic function (ie. devoid of synaptic connections). Warr[50] studied the parallel nature of this portion of the brainstem. He proposed a pathway to the inferior colliculus that contained synaptic connections. The superior olivary complex and the lemniscal nuclei being possible synapse locations.

The Ponton *et al.*[47] model indicates that the asynaptic pathway between the cochlear nucleus and lateral lemniscus is responsible for the III-IV IPL. Furthermore, that the asynaptic nature means that this IPL parameter is free from synaptic delay and thus is representative of axonal conduction velocity only. They stipulated that wave V is generated by the axons that synapse in the medial olivary complex on their way to the contralateral lemniscus. It would follow that the III-V IPL will represent axonal conduction and synaptic delay. Subtraction of the III- IV IPL from the III-V IPL (ie. the IV-V IPL) will be a measure of only the synaptic delay present between waves III and V. Ponton *et al.*[47], thus, suggest that the III-IV IPL is used to measure axonal conduction with the IV-V IPL being indicative of synaptic delay.

This current research is concerned with the data from the preterm population. The use of the wave IV parameter with this population is not practical. Wave IV detectability in very young infants tends to be low and thus not suited for studying maturational characteristics at early PCAs. This data would be limited and potentially unreliable.

Ponton *et al.*[47] supports their theory concerning synaptic interaction by citing studies of ABRs recorded intrasurgically. Direct electrical stimulation on cochlear nucleus neurons generates waves that correspond to waves III, IV and V of the acoustically stimulated ABR. They cite the relative behaviour of waves IV and V to an increase in stimulus rate. With an increase in stimulus rate from 100 to 200pps, wave V is attenuated significantly with wave IV being unaffected. They conclude that the two waves are generated by different pathways. The interpretation is that the rate sensitive pathway is responsible for wave V and contains a synaptic junction. The rate non-sensitive wave IV is thus produced by a parallel asynaptic pathway.

With the data from this current research, measurements are made with varying stimulus rates for the wave III and V parameters. It can, thus, be stipulated that measurements of the III, V and I-V IPL at low stimulus rate will represent axonal conduction and synaptic delay. Thus, the III-V IPL parameter represents one of the parallel contralateral pathways. This current research measures only one of the parallel pathways that are present. Measurements at higher stimulus rates will cause an increase in the III-V IPL value due to rate attenuation of the synapse present. It thus follows that if the base stimulus rate data is subtracted from the data at the higher stimulus rate, an index of delay caused exclusively by synaptic function can be acquired.

It is thus proposed that low stimulus rate (13pps) data is indicative of a basic function (including axonal conduction and underlying synaptic delay due to normal function) and the difference between high and low rate data (37-13 and 61-13pps) is indicative of an exclusively synaptic delay. Furthermore, that this delay is linked to the synaptic efficacy. The exclusion of the basic synaptic delay from low rate data is not possible with the age group and thus the data that was collected. Therefore, the low rate data investigates the neural transmission time for basic auditory function with the increase in this time being an index of synaptic efficacy.

Ponton *et al.*[47] also apply a similar methodology to the peripherally generated I to III waves. It is accepted that wave I is generated by axons leaving the cochlear and wave II along the auditory nerve. Wave III is generated by axons leaving the cochlear nucleus. It therefore follows that the I-II IPL is indicative of axonal conduction in the auditory nerve with no synaptic junctions. The II-III IPL will contain axonal conduction of the auditory nerve and, because wave III is generated post cochlear nucleus, a synaptic delay component. These two IPLs are sequential. It follows that the I and III waves collected for this current research will provide information (I-III IPL) on the neural transmission time (including axonal conduction and basic synaptic delay) in the region from the distal end of the auditory nerve and cochlear nucleus. Again, rate attenuation will affect the synaptic component contained within this IPL region.

The first ABR wave (wave I) is generated after the hair cells in the distal end of the auditory nerve. The transmission time for wave I is due to the time for the sound energy to traverse the structures of the ME and a cochlear transduction component. There will also be a delay from the basic function of the synapses within the hair cells before the primary auditory neuron. Any rate attenuation affect of the wave I latency is due to the effect of the hair cell synapses. Again, subtraction of low stimulus rate data from higher rate data will provide an index of the synaptic efficacy of the synapses in the hair cells.

1.6.1 Rate Effect

Following stimulation of an auditory nerve fibre by an acoustic stimulus, its response to subsequent stimuli will initially be depressed (the process of adaptation). There is a period of time until the nerve fibre recovers to its unadapted state. Terkildsen *et al.*[51] suggested that this adaptation results in a decrease in the number of active neurons and their synchronization. It is not clear whether this process takes place in the cochlea or along the auditory pathways. Don *et al.*[52] reported that the refractory period of neurons, changes in synaptic efficacy or cochlear transduction may be involved. Lina-Granade *et al.*[46] suggested that cochlear transduction and synaptic efficacy may be responsible for the maturational changes in the rate effect. They suggested that neural refraction is not involved due to the stimulus intervals being far greater than the neural

refractory period of 1ms[45]. Ken-Dror *et al.*[45] reported that the role of nerve conduction (associated with axonal diameter and myelination) is not affected by rate. Therefore, subtraction of a latency at higher rate from one of a lesser rate will eliminate such properties.

Lina-Granade *et al.*[46] reported that only wave V, and not wave I, displays significant changes in the rate effect with age. They suggested that this implies that maturation of the mechanisms of adaptation are predominantly at a central level, with the cochlear role not altering with age. They concluded that maturation effects are dominated by central synaptic efficacy. There is also significant evidence that wave III is also adapted by rate, albeit less than for wave V. Lina-Granade *et al.*[46] suggested that there is an accumulative effect progressing through the auditory system. This would be compatible with a dominating synaptic role in the developmental differences of the rate effect. The neural generators of ABR waves suggested by Moller and Janetta[53] will be used for discussion. Wave I is generated by the cochlea nerve near the inner hair cells, wave III from the cochlea nucleus, and wave V from the lateral lemniscus near the inferior colliculus. According to Moller and Janetta[54], there are synapses between the inner hair cells and the primary neuron, in the cochlear nucleus, in the superior olivary complex and in the lateral lemnicus. It could, therefore, follow that wave V is more sensitive to adaptation due to the generator lying three or four synapses through the system. There are two synapses preceding the wave III generator and only one synapse below the wave I generator. Lina-Granade *et al.*[46] suggested that higher stimulus rates could identify a wave I rate-induced shift. This would represent the maturation of the synapse between the inner hair cells and the primary auditory neuron.

These findings suggest that central synaptic maturation is dominant in the maturational changes seen with adaptation. Some previous studies[55, 56] have suggested that the cochlear mechanisms show maturation in the first months of extra-uterine life. Presently, the physiological processes surrounding adaptation have not been confirmed. However, it is agreed that the latency-rate relationship is useful in assessing neurological function. The assumption of a predominantly synaptic interaction will be used.

Jiang *et al.*[57] studied the rate effect for term infants and compared the attenuation observations to adult data. Overall maturation is made up of fibre maturation and

synaptic maturation. Low stimulus rate is representative of general function. Rate attenuation is primarily representative of neural processes concerned with the efficacy of synaptic transmission. In addition, that the rate attenuation is also concerned with neural synchronization and metabolic changes following a physiological challenge to auditory neurons[58]. Jiang *et al.*[57] wanted to investigate whether the differences found with low stimulus rate neonate data and adult data was repeated for rate attenuation characteristics. That is to say, whether general function and synaptic efficacy of the neonatal brainstem are equally immature when compared to the adult. They found, as previously reported, that the neonatal group had high levels of rate attenuation. Parthasarathy *et al.*[59] and Lasky[60] supported this theory with wave V latency shifts being greater for neonates than in the adult population. This shows a lack in the ability to process rapid acoustic stimuli. Jiang *et al.*[57] suggested a more profound difference in L-R function slope between neonates and adults for wave V rather than waves I and III. However, waves I and III gradients were higher than adult values. This would support the behaviour suggested by Lasky[61] who found L-R functions for waves I and III to be similar to those found in adults whilst wave V gradients were higher for neonates. Jiang *et al.*[57] also analyzed percentage rate attenuation to investigate the effect of low stimulus rate maturity between neonates and adults. They found that percentage increases were also higher than adult percentage values. They concluded that this shows that synaptic efficacy of the neonate is relatively less mature than the adult.

Eldridge and Salamy[62] studied the rate effect in the preterm infant between 32 and 45 weeks PCA. They also found rate attenuation to be greatest for wave V and least for wave I, with younger infants displaying greater attenuation for waves III and V for younger infants. Despland and Galambos[63] found twice the amount of attenuation occurring for wave V over wave I. They concluded that half the net rate attenuation occurs at the cochlear level and half within the brainstem. Eldridge and Salamy[62], however, found a three-fold increase in rate attenuation for wave V over wave I. Examining the I-III and III-V IPLs, they found that approximately 3/4 of the contribution was located in the III-V IPL region for infants prior to term. For their term group, just over half the contribution was in the III-V IPL region.

1.6.2 Path Length and Axonal Diameter

Whilst myelination increases the conductile neural properties, there are other possible factors affecting the reduction in ABR latencies with increasing age. Obviously, as an infant grows so head size will increase resulting in an increase in the length of the auditory pathways. In addition, it is also recognized that axonal diameter also alters with growth. Moore *et al.*[64] studied ABR conduction times and correlated them with a reconstruction of the auditory pathways from postmortem foetal data. They studied infants ranging from the preterm period up to 1 year of age. For this current research, changes from the early preterm period through to term are of interest. Moore *et al.*[64] suggested that more than one aspect of the myelination process is responsible for the apparent increase in conduction time in the auditory pathways. The fact that reductions in conduction time are observed during the preterm period suggests that any increase in path length is more than balanced by changes in axonal structure or process characteristics.

It has already been stated that generally reductions in ABR latency at low stimulus rate are predominantly due to increased thickness of myelin around the axon. An increase in axonal diameter due to the development of the infant is also indicated. Colello[44] reported that these two processes are connected with myelin forming oligodendrocytes displaying control over increases in axonal diameter. Moore *et al.*[64] commented on the previous research into increasing peripheral nerve length and its affect on conduction velocity. They reported the behaviour of axonal lengthening where internode distances increase with the number of nodes on the axon remaining the same. Thus, the saltatory conduction behaviour of the axon would make the conduction velocity independent of internodal length. It would, therefore, be possible to conclude that increasing length of auditory pathways would not affect ABR latencies.

1.7 Anatomical Maturation Processes - Foetal AEP Studies

The study of prenatal hearing development has had little attention. In order to better document the maturational processes in the premature auditory system, a number of animal studies on foetal ABRs have been undertaken. These studies can give an idea

of the sequence of events in maturing neonate auditory systems. They tend to concentrate on the recording of ABRs. Knowledge of middle latency response (MLR) and slow vertex potential (SVP) recordings in the late foetal and preterm period of life is still limited.

Pierson *et al.*[65] studied foetal sheep for ABRs. Hearing sensitivities of human and ovine are also similar. They found that the earliest identifiable ABR was at 111dGA[c] (normal gestation is 145 days), a full set of peaks were observed. It was found that ABR thresholds improved rapidly from 111 to 123dGA. Pierson *et al.*[65] suggested that a number of events could give rise to this rapid development; increase in the number and activity of auditory pathway synapses, changes in basilar membrane response, and development of endocochlear potential which is necessary for hair cell activity. Threshold development was found to be less rapid from 123 to 136dGA, this development was put down to further maturation of the cochlea and brainstem auditory pathways. Salamy and McKean[16] suggested that in humans the peripheral and central auditory pathways matured at different rates. Pierson *et al.*[65] found all waveform peaks improving from 111 to 136dGA; increased myelination and axon diameter, and improved neural synchronization decreasing peak latencies. The latencies of waves I and II were seen to decrease less than waves III and IV.

The ABRs represent neural processes. They are, therefore, dependant upon neural maturation, in particular the myelination and synaptic functional development. Cook *et al.*[66] studied ABRs from foetal sheep, they suggested that exact matching of ABR waveforms to single neural structural origin may not be possible. They found that the waveforms were first observed at 117dGA, the appearance of the ABR being dependant on the wave I region. This represents maturation in the cochlea. It is also possible that ME properties, such as ossicle control, may have an influence. Stimulus rate effects were seen for waves IV and V, these peaks being generated at higher CNS levels. They suggested that the rate effect reflects the greater number of synapses forerunning generation of these waveforms.

Wang *et al.*[67] recorded ABRs from foetal guinea pigs at 7 weeks GA (term being 8 weeks). Traces were found to have clear waves I, III and V at high intensities. A reference study was carried out on term newborns, it was seen that III-V IPL changed

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[c] Refers to days of gestational age.

markedly, with little change in the I-III IPL. They supported the view that peripheral auditory structures mature earlier and undergo little change in late gestation and after birth. They also suggested that the higher regions of the brainstem mature more rapidly during the same time period. Salamy and McKean[16] reported on a human study with preterm newborns. They indicated that the ABR can be identified from 26 to 28 weeks PCA. They suggested that changes in the ABR are initially reflected as changes in the auditory nerve and cochlear nuclei (waves I and II). After 34 weeks PCA, waves I and II show little change. The maturation occurs in the upper regions of the brainstem and midbrain (waves III, IV and V).

1.8 Infant ABR Characteristics

Infant ABR characteristics are largely dependant on age. The preterm infant commonly displays responses at 27 to 28 weeks PCA. However, it is possible that responses will not be obtained until 30 weeks PCA or later. Higher stimulus intensity levels are often necessary, with the response being relatively low in amplitude[68]. It has been demonstrated that the alterations in latency, amplitude and morphology are related to physiological immaturity[69]. It is well known that the infant response displays waves I, III and V. Latency and amplitude values differ from adult norms[69] due to the immaturity of the auditory system. This will delay the absolute and interpeak latencies of the three waves mentioned.

Cevette[70] reported a decrease in mean wave V latency and I-V IPL of 0.1 to 0.2ms/week through to term. Cevette[70] suggested that wave I will reach adult values by 6 months, wave V and the I-V IPL being 'adult-like' by approximately 16 months. Gorga *et al.*[71] obtained normative data for preterm infants. They found that the I-III IPL was longer than the III-V IPL, similar to adult results. Decreases in IPLs with increasing age are small but systematic, the most apparent being the I-V IPL. They also reported that standard deviations (S.D.) across the age groups were similar, this suggesting normal distributions. Murray[72] reported that the most rapid maturation occurred between 34 and 36 weeks PCA, the greatest shift in IPL occurring between 36 and 38 weeks PCA. Wave I was found to be similar to that of adults at term, the later components (waves III and V, and the I-V IPL) having slower maturation completed by 1.5 to 2 years of age. They

concluded that the central transmission time (I-V IPL) is the most useful tool in assessing CNS function. Krumholz *et al.*[73] found reproducible I, III and V waves in nearly 100% of 30 week PCA infants, with waves II and IV being present in 60 to 80% of 39 to 43 week PCA subjects. They reported that the greatest decrease in latency of immature infants was between 28 and 33 weeks PCA. The interpeak latencies (I-III, III-V and I-V IPLs) also reduce during the preterm period and are more variable than term newborns and adults. Tudehope *et al.*[74] reported no difference in wave V or IPL measures at 70dB nHL between very low birthweight infants (LBWI) and a normative group of infants at term. However, particular medical conditions found with preterm infants and prematurity itself have been reported as affecting the ABR[75].

It has been reported that the smallest ABR amplitudes are found in neonates and the largest in infants, the amplitudes of adult waveforms being in between[76]. Lieberman *et al.*[76] found that absolute wave V amplitudes are smaller than adults. Wave I amplitude, however, can be as much as twice that of adults, this leading to a reduced I-V amplitude ratio. Lieberman *et al.*[76] indicated that close electrode approximation to the auditory nerve, due to infant head size, may cause the greatly increased wave I amplitude. Recording procedures, transient changes in peripheral sensitivity, and presentation levels have been suggested as factors increasing the variability of absolute amplitude readings. These factors have limited the use of amplitude measurements in infant ABR assessment. However, amplitude ratio's are used in adult assessment, wave I reflecting peripheral activity and wave V central activity. Typical ratio's for infants approximate to 1, commonly 3 for adults.

Krumholz *et al.*[73] reported that relative wave amplitudes mature with advancing age. They implemented accepted standard measures used in adults and older children to assess infants near term. They found relative amplitude values to be stable and potentially useful, although the number of premature subjects in their study was too small to draw conclusions. Eggermont and Salamy[77] studied the I-V amplitude ratio's in preterm infants as a tool for diagnosing abnormal development. They saw a general increase up to 100 weeks PCA, followed by a decline. Jiang *et al.*[78] commented on the lack of a consistent trend for ABR amplitude values due to the substantial variability involved. They reported that the I-V ratio was more stable than the absolute amplitude values, the ratio increasing until term. However, they also found the absolute value of wave V to be

superior to the I-V ratio in some clinical applications. The absolute amplitude values of waves III and V were found to be greater in females after 3 years. They concluded that amplitude was strongly dependant on age, although physical build also had an affect (thinner subjects with smaller head size had increased amplitude values).

Data collected at low stimulus rate has been used to represent the nerve transmission properties in the various parts of the auditory system. It is generally accepted[73, 42, 79, 80] that nerve transmission properties are strongly linked to myelination. Eggermont[43] used the assumption that maturation of auditory structures (as reflected in ABR latency measurement) is the result of an interaction between increased myelination and increased neural length. The preterm infant is known to suffer from delayed myelination. This delay is absolute. A number of studies[81, 82, 29] have reported on the neonatal brain with MRI. They found a lesser extent of myelination in the preterm than the term infant.

Moore *et al.*[42] reported that myelination occurs from 26 weeks PCA onwards, and that definitive myelination is present in all auditory pathways by the 29th week. They reported that beyond the 29th week PCA, myelination density is increased until at least 1 year of postnatal age. Moore *et al.*[42] concluded that this increase in density is the predominant factor in the steady decrease in the latency of the ABR waves III and V. The intensity of myelin staining in the cochlea nerve (wave I) and the brainstem (waves III and V) has been reported as increasing steadily during the 30 to 40 week PCA period[83, 84]. There is, thus, a relationship between the increased density of myelination and the time course of shortening ABR latencies during the preterm period. This behaviour has been reported as being greater in the preterm infant (between 28 and 40 weeks PCA) than in the term infant after birth[85]. Term infants show a slower maturation of IPLs which steadily approach adult values. Eggermont[43] reported that the PAS wave I is conductively mature in the 45th week PCA for the term infant. The myelination process has been reported[84] as progressing in a centripetal direction through the auditory system. This suggests that the later ABR components would be more affected by delayed myelination in the preterm infant[86]. It would, therefore, follow that myelination would occur in the PAS, progressing to the auditory nerve, with the lower brainstem region being the last to develop. For this current study the auditory system is divided into the

PAS, the auditory nerve and the lower brainstem regions (I-III and III-V IPLs respectively).

The physiological aspects of the rate effect have generally not been discussed in previous research. Jiang *et al.*[87] used the rate effect to assess synaptic efficacy in children and adults. However, their discussion did not go beyond the term "synaptic efficiency". As previously mentioned, the physiological discussions relating to evoked potentials tend to concentrate on axonal myelination[42, 87]. Pujol and Hilding[88] reported the presence of myelin sheathed axons within the cochlea several days before the onset of recordable action potentials. They speculated that this delay was due to synaptic immaturity. Moore *et al.*[42] mentioned synaptic efficacy as being an additional factor affecting central axonal conduction velocity. Mercuri *et al.*[86] reported on myelination in relation to evoked potential recording. Whilst their study does not mention the maturational time course of synaptic efficacy, they do suggest that axo-dendritric connections (synapses) start to maturate at the same time as the myelination of the auditory nerve (24-26 weeks GA). The possible direction of synaptic efficacy maturation is not considered (centripetal direction for myelination). It would appear that the physiological processes of synaptic maturation is an area requiring further research.

It is generally agreed that there is a reduction in the rate effect during the preterm period[45, 61]. Data tends to concentrate on the L-R function of the absolute latency of wave V. Studies of the rate effect on IPL parameters are more limited. Lina-Granade *et al.*[46] studied the rate effect on various parameters in the preterm period. However, a discussion of maturation was not presented. They presented data in four time categories over the preterm period. Data for waves I and III, and the I-III and I-V IPLs display inconsistent maturational characteristics. Only the III-V IPL and the absolute latency of wave V show consistent reductions in the rate effect. Only the wave V reduction was found to be statistically significant.

1.9 Physiological Model

- Wave I is generated at the start of the auditory nerve near the outer hair cells (OHC). The region before the wave I generator will be referred to as the peripheral auditory system (PAS).
- Wave III is generated by axons leaving the cochlear nucleus.
- Wave V is generated by nuclei of the lateral lemniscus.
- The I-III IPL represents the properties of the auditory nerve.
- The III-V IPL represents the properties between the cochlear nucleus and the lateral lemniscus. This will be referred to as the lower brainstem region.
- The I-V IPL represents the properties in the auditory nerve and lower brainstem region.
- Base stimulus rate (13pps) data represents the conductive properties of the PAS and the neural transmission properties beyond the PAS.
- The main PAS conductive components are middle ear (ME) conduction, cochlear transduction and basic synaptic delay.
- Neural transmission is governed by myelination status and basic synaptic delay. Basic synaptic delay is latency caused by the activation of synapses.
- The rate effect (increasing on the base stimulus rate, 37 or 61pps) will introduce an additional delay caused by rapid repeated stimulation of synapses. Subtraction of the base stimulus rate data will eliminate the transmission properties from the rate attenuated data. This will be termed the rate shift. The rate effect will be used as an assessment of synaptic efficacy.
- The interaction of intensity on latency to produce a latency-intensity (L-I) function will be used as an assessment of neural sensitivity.

1.10 GLOSSARY

altricious - an animal born without behavioural auditory function

brachium - of or relating to an arm

cation - an ion carrying a positive charge owing to a deficiency of electrons; in an electrochemical cell cations migrate toward the cathode

caudal - of the posterior part of the body (posterior)

distal - situated away from the centre of the body or point of attachment

dorsal - in a higher position (superior)

ducussate - cross to the opposite side

ectodermal - outermost layer

fenestra - small hole or opening in a bone, etc. especially one of two in the inner ear

lateral - of, at, towards, or from the side or sides. A side part etc., especially a lateral shoot or branch

medial - situated in the middle

medulla - continuation of the spinal cord within the skull, forming the lowest part of the brainstem

manubrium - handlelike structure or part

mesenchyme - the meshwork of embryonic connective tissue in the mesoderm from which are formed the connective tissues of the body

neuroglia - connective tissue supporting the CNS

nasopharynx - the part of the pharynx (throat) that lies above the level of the soft palate. Communicates with the auditory tube

phalanx - referring to a line, row or array

pons - part of the brainstem that links the medulla and the thalamas

precocious - an animal born with behavioural auditory function

ramification - subdivision of a complex structure or process comparable to a tree's branches

rostral - nearer the front (anterior)

tensor palatini - muscle pertaining to the palate

ventral - of the front or lower surface (inferior)

Figure 1.1 Cross-sectional view of the human ear (Courtesy of Abbott Laboratories)

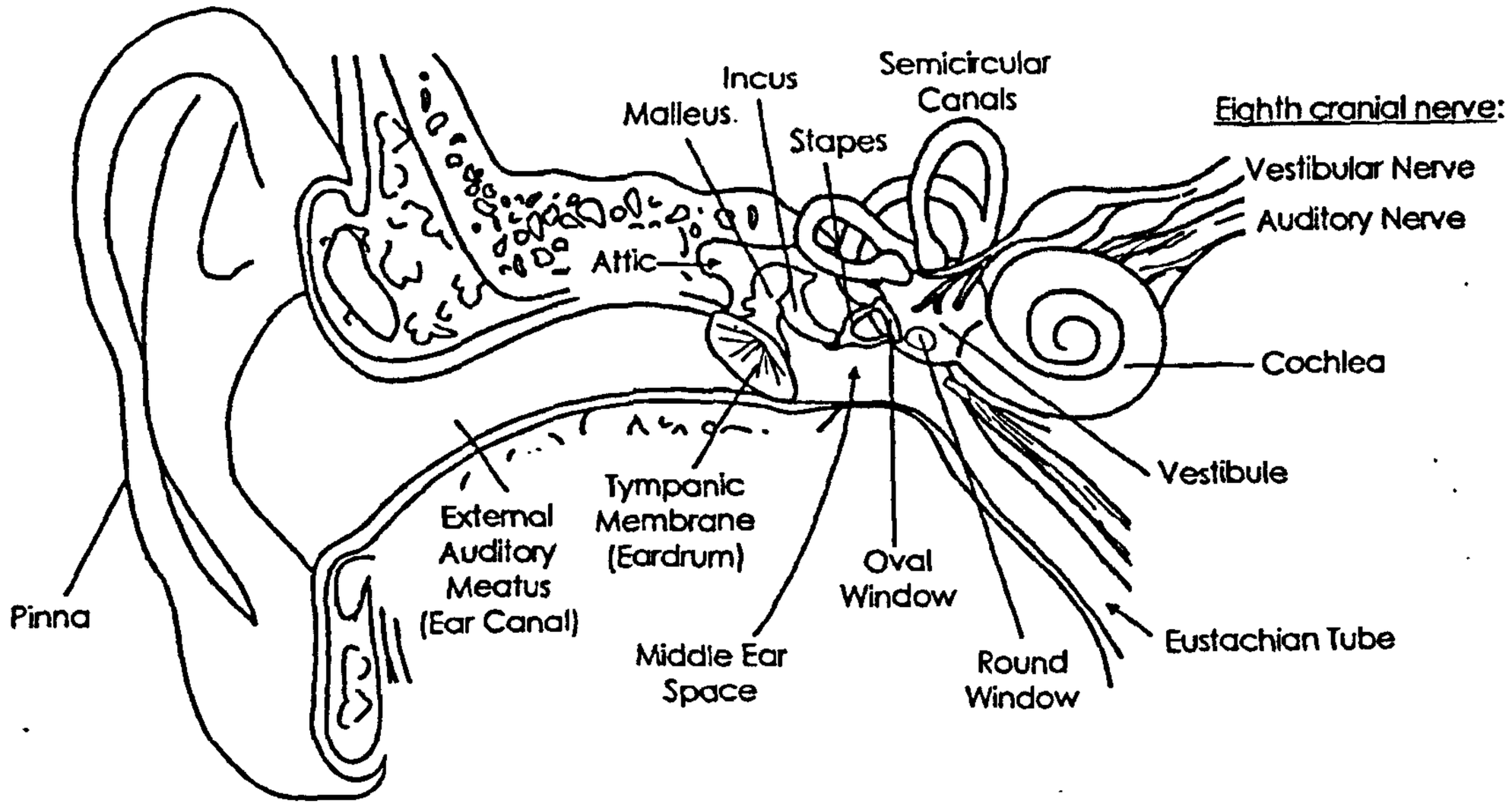


Figure 1.2 Schematic representation of the ossicular chain (Courtesy of Thieme Medical Publishers)

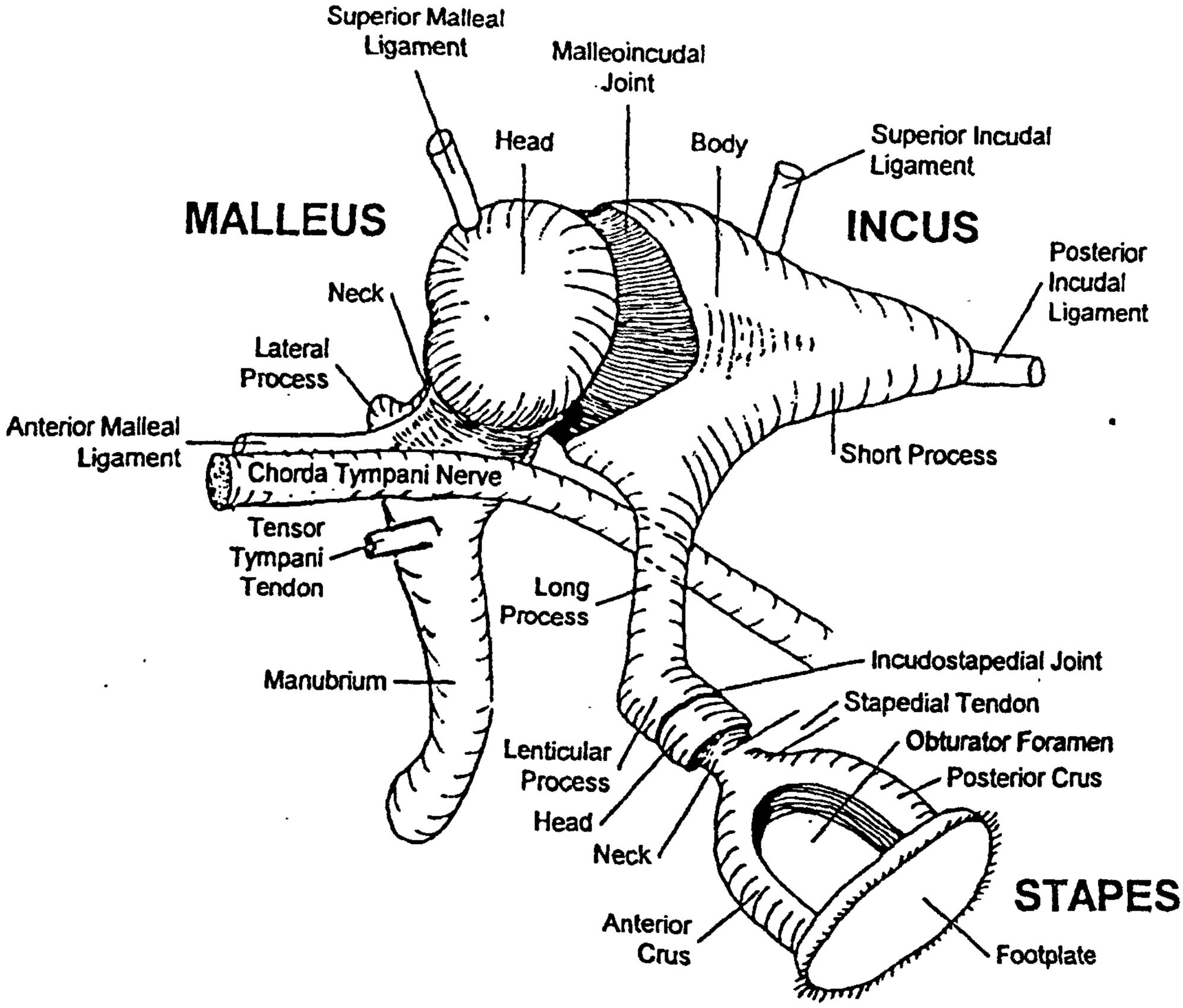


Figure 1.3 The human cochlea (Courtesy of Thieme Medical Publishers)

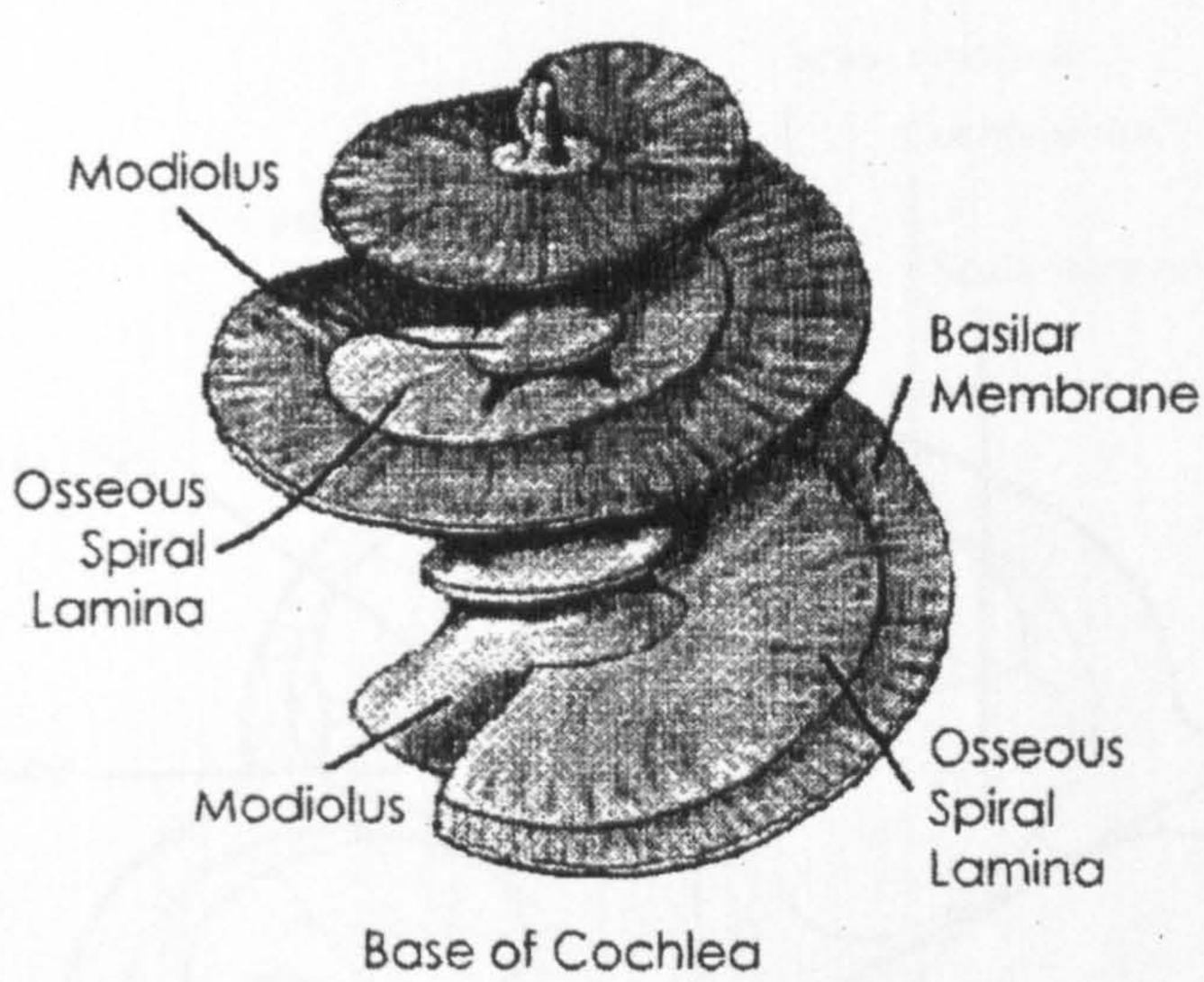


Figure 1.4 Section through the cochlea (Courtesy of Lippincott-Raven Publishers)

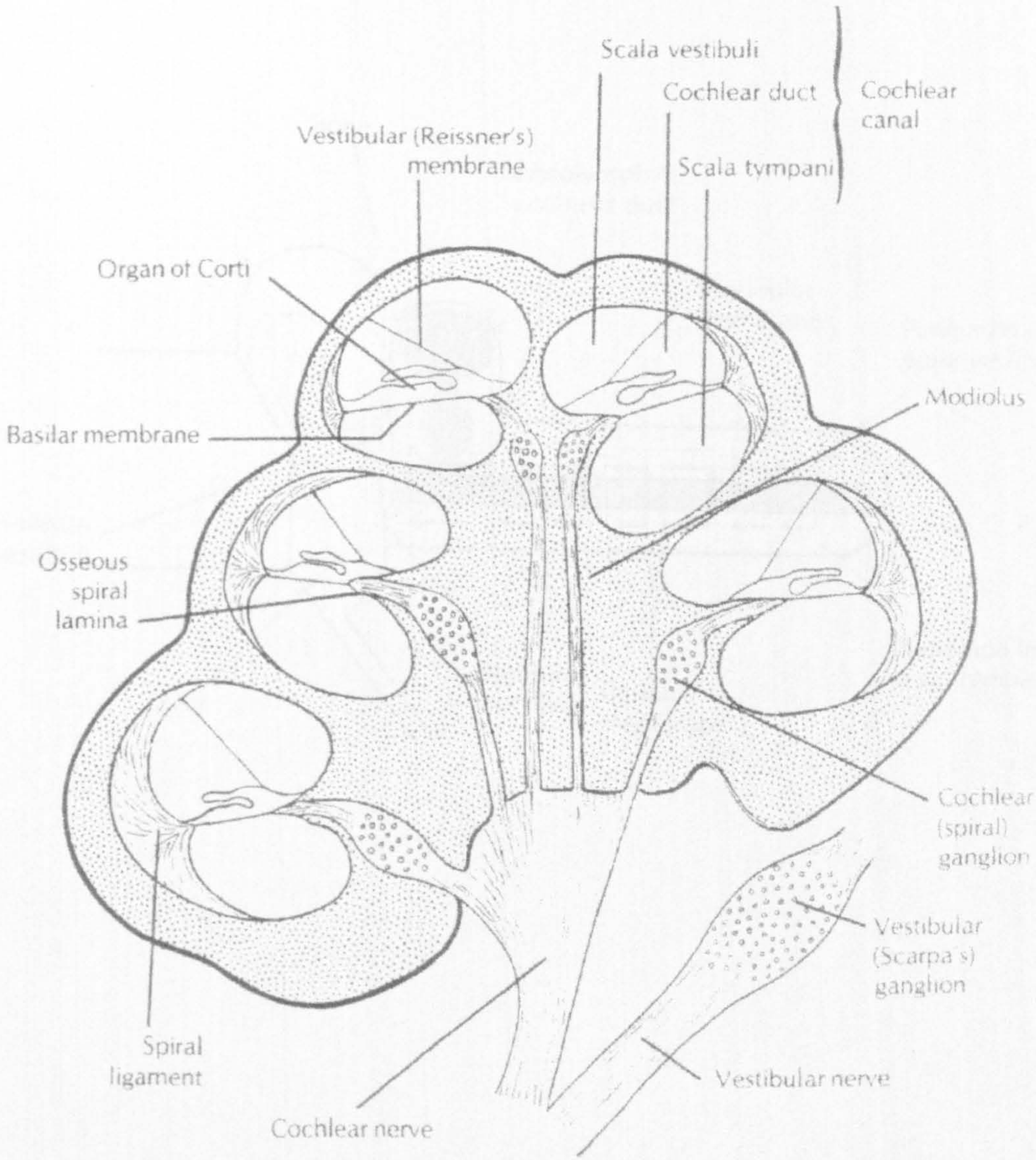


Figure 1.5 Schematic representation of the manner in which sound waves in the perilymph and endolymph cause vibration of the basilar membrane (Courtesy of Lippincott-Raven Publishers)

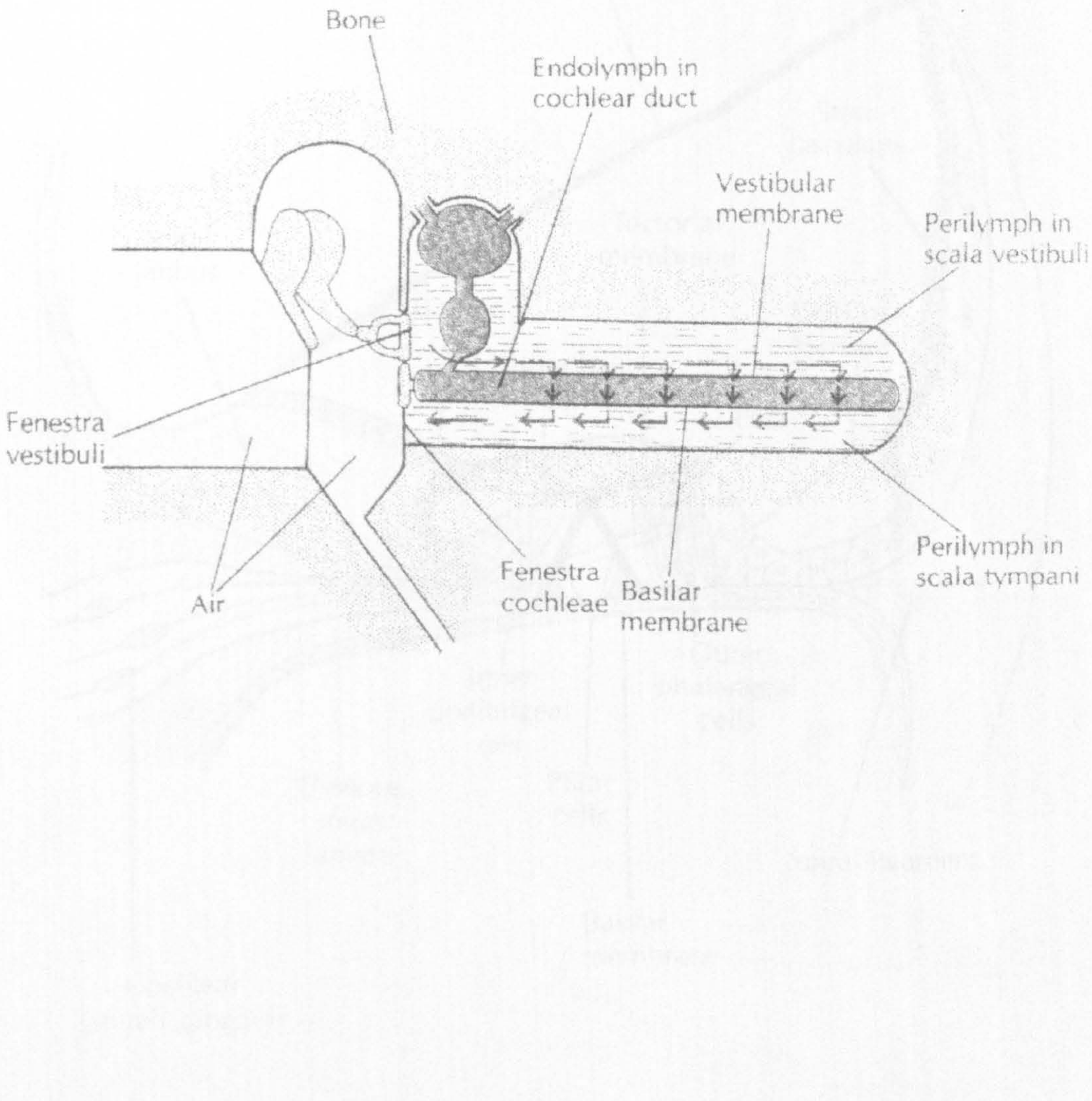


Figure 1.6 Structure of the cochlear duct and the spiral organ of Corti (Courtesy of Lippincott-Raven Publishers)

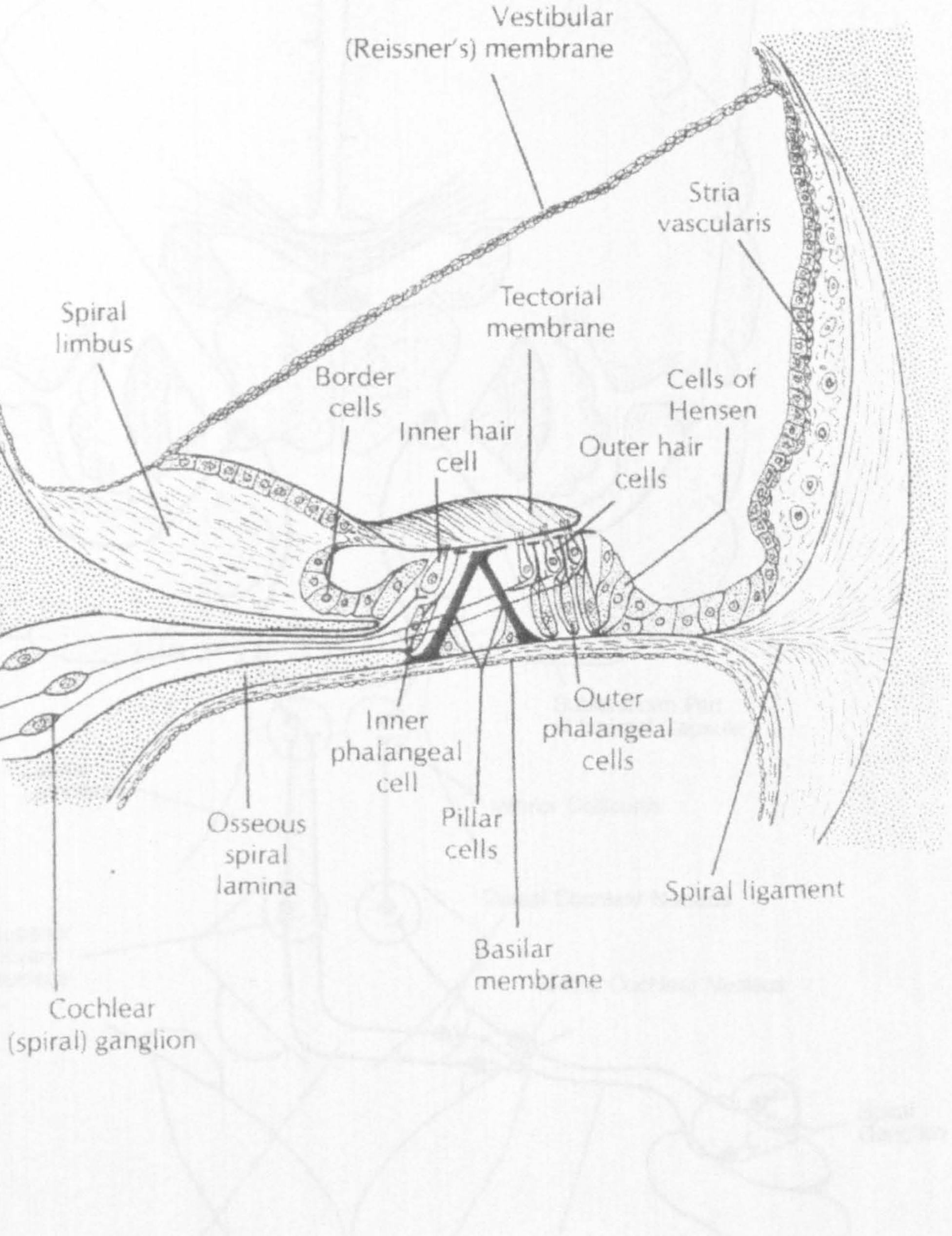


Figure 1.7 Ascending auditory pathway (Courtesy of Philadelphia Publications)

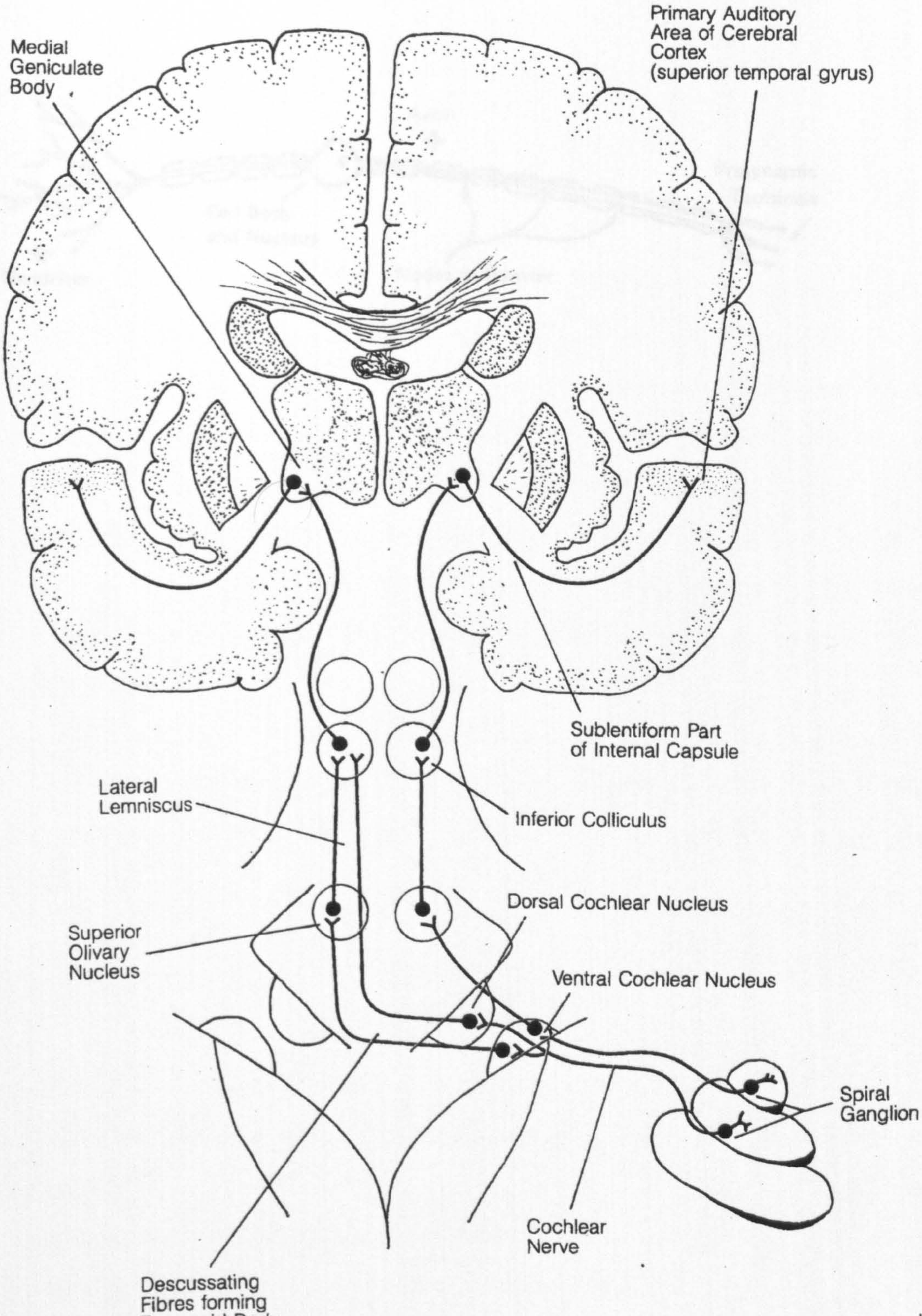
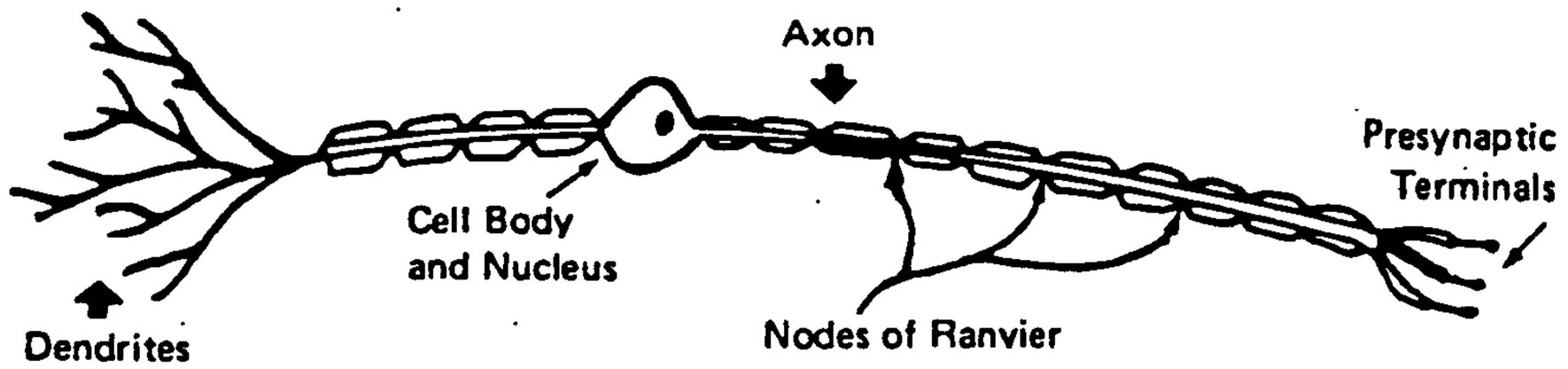


Figure 1.8 Simplified representation of a typical human cell (Courtesy of Thieme Medical Publishers)



CHAPTER TWO

AUDITORY BRAINSTEM RESPONSE (ABR)

2.1 Introduction

This literature review examines the techniques available for recording reliable and accurate ABRs from the neonate population. This is especially important for data collection from preterm infants in the Neonatal intensive care unit (NICU) environment. Collection parameters for the Bio-Logic Navigator evoked potential machine are investigated with reference to the use of this machine in the NICU environment. A review of test protocols is presented to ensure the collection of the optimum amount of relevant data for analysis. Various wave component marking criteria for the neonate ABR morphology are assessed. Strict marking criteria are essential for consistency and reliability of data.

Suitable collection parameters and a comprehensive test protocol are suggested. Initial testing is performed on both term and preterm infants to validate the conclusions of the review. These tests are briefly presented with the modifications that were necessary.

2.1.1 Specific Deliverables

- To define suitable signal filter settings for testing in the NICU.
- To determine the appropriate number of sweeps in order to obtain reliable wave morphology.
- To establish a repeatable pattern of electrode placement.
- To identify a suitable (and realistic) electrode impedance limit.
- To identify an appropriate stimulus rate for standard testing and to assess the usefulness of the rate effect.

- To analyze the intensity interaction on ABR data and to establish criteria for threshold estimation.
- To investigate the possible use of frequency specific stimuli.
- To identify and test a reliable wave marking criteria.

2.2 Types of Auditory Evoked Potential (AEP)

There are various types of AEP that can be recorded. Potentials[a] originate in the auditory nerve (compound action potential (CAP) and ABR waves I and II), the auditory brainstem (ABR waves III to V), the auditory midbrain and thalamus (ABR waves VI and VII) and the auditory cortex (middle latency response (MLR) and the slow vertex potential (SVP))[1]. The latency of AEPs is dependant on how rostral the origin (or generator) site is in the auditory system. In adults, the CAP (and ABR wave I) has a latency between 1.5-2ms, whereas the wave V (thought to be generated in the lateral lemniscus) has a latency greater than 5.5ms. The MLR, which consists of one positive peak (P_a) with negative deflections (N_a, N_b) either side, has a positive peak latency of approximately 25ms. The SVP, consisting of two positive peaks (P_1, P_2) and two negative deflections (N_1, N_2), has a latency of approximately 90ms for the first negative deflection (N_1). As stated, there are three main regions of AEP response; ABRs and the two cortical potential regions, MLR and SVP.

The equipment utilized in this study has the capability of recording the ABR components of the AEP. The advantages of using these components are that they are recognizable from very early ages (28 weeks post-conceptional age (PCA)), the results are reliable, and the changes in component characteristics have significant neurological implications. The ABR waves are labelled using Roman numerals (I-VII). The marking criterion of these waves will be discussed later in this chapter.

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[a] Acoustic stimuli travelling through the auditory system result in electrical potentials which can be recorded from the scalp. These potentials are produced by the structures of the auditory system. It is not known exactly which anatomical structures are responsible for the peaks observed in the ABR data.

2.3 Collection Parameters

2.3.1 Electrode Location

The measurement of the ABR from the scalp requires the acquisition of potential differences rather than absolute values. A recording montage of a single electrode would leave the ABR signal undetectable due to the relative size of the signal to the noise components. The detectability found when using two or three electrodes on different sites on the head relies on the fact that many of the noise components are the same at all points on the head. These noise components include physiologic potentials, such as cardiac and myogenic, as well as non-physiological potentials. Thus, by taking differential measures some of these noise components will be potentially cancelled out. This is the initial step in improving the signal-to-noise ratio (SNR). It is therefore beneficial to locate electrodes on sites where the ABR signal differs but where the noise is similar.

Standard AEP acquisition requires the placement of three or four electrodes on the subjects scalp. One electrode is used as a ground with the remaining two (or three) being connected to the preamplifier inputs. In this case we are concerned with the three electrode configuration. These electrodes are commonly referred to as the "active", "reference" and "ground"; these definitions can be misleading as they do not accurately represent the underlying physiological events. More accurate descriptors include, "positive" and "negative", relating to electrode input at the preamplifier stage; and "noninverting" and "inverting" referring to the amplifier function. The third electrode, the "ground" or "common", serves as a reference electrode for the other two. Unlike the similar field of electroencephalography (EEG), there is not a general consensus on the optimum placement of electrodes for AEP recordings. However, there is the International 10-20 system identifying commonly used electrode locations (fig. 2.1). Jasper[2] originally proposed a design for standardizing electrode placements for routine clinical EEG. Electrode placements are measured from four standard positions on the head; the nasion, inion, and the left and right auricular sites[3]. The advantage of this system over other methods is that it is based on individual measures of head circumference and anatomical landmarks. This results in a reasonable relationship

between electrode position and brain structure. However, clinical interpretation of EEG traces is still restricted to the level of lobes and hemispheres.

Electrodes for AEP testing are arranged such that electrical potential difference is measured between pairs of electrodes on the scalp. The active (positive) electrode is placed on either the vertex (C_z) or high forehead (F_z). The reference (negative) electrode is placed on a site assumed to be inactive (neutral), such as the ipsilateral[b] earlobe (A_1, A_2) or mastoid (M_1, M_2). The ground electrode is the contralateral equivalent (earlobe or mastoid). Research suggests that the most acceptable compromise for the positive electrode is on the vertex (C_z). The parietal site (P_z) has been proposed as the optimum position for measurement of all wave components of the ABR. However, many clinicians use the high forehead (F_z) location for ease of testing.

It must be noted that variations in electrode placement will affect wave morphology, latency and amplitude measurements. Utilizing the earlobe instead of the mastoid site for the reference electrode can significantly alter the amplitude of wave I. Wave I amplitudes can be increased by using a horizontal montage (mastoid to mastoid) over the vertical montage (vertex to mastoid). In adults, for maximum wave V amplitude, the vertical montage (vertex or forehead to mastoid) is most efficient. In infants, this is not always the case, with as many as 40% displaying greater wave V amplitudes in the horizontal montage[4].

Unlike negative electrode sites, changes in amplitude and latency between vertex and forehead have not been well documented. Clarke-Cox[5] reported an 18% reduction in wave amplitude when using an high forehead position just below the hair line as opposed to the standard vertex position. Recent studies[6, 7, 8, 9] have cited the positive electrode on the high forehead, this position being more practical for use in neonatal testing, and well documented by other studies. Initial testing for this study has recorded ABRs using the high forehead (F_z) location for the positive electrode and the mastoid (M_1, M_2) for the reference site. It was found that variation of electrode placement was necessary to achieve good impedance. This was especially true for the forehead electrode, in some cases a low forehead location was required. This would question the use of amplitude analysis for this study.

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[b] Ipsilateral and contralateral refer to the same side of, and the opposite side of the body respectively.

2.3.2 Electrode Impedance

A good electrode-patient interface is critical in obtaining good evoked potential (EP) readings. This interface acts as a complex circuit but can be simplified to a resistor and capacitor in parallel. The impedance to current flow is a good measure of the condition of the interface. A test current of a few millivolts is sent through one interface with the patient and is measured at another electrode. The measured drop in voltage is proportional to the impedance at the two electrode sites. An alternating current is implemented because the interfaces have polarizing properties. The frequency of this current should be lower than the frequency of the signal to be used for the EP measurement. This process of impedance measurement is vital in acquiring reliable ABRs.

It is generally accepted that ABR electrode impedance levels should be below 5kΩ for reliable data collection. However, this value is not always achievable. Some reports have suggested that a limit of 10kΩ would be more practical and still clinically acceptable[10]. Eccard and Weber[10] also reported that impedance balance is a more critical factor when looking at the reliability of data. Unbalanced impedances can be considered more detrimental to the accuracy of ABR data than higher individual impedances. An appropriate criteria could require well balanced impedances below 10kΩ, aiming for less than 5kΩ.

2.3.3 Signal Amplification

Activity from the electrodes is first passed into a differential amplifier in order to assess the differential nature of the recording and to amplify the activity for further manipulation. The differential amplifier subtracts activity at the inverting electrode from the non-inverting electrode. The amplifiers electrical reference is connected to the common electrode. The gain value is used to amplify the inverting non-inverting differential (gain of 50,000 to 300,000).

The differential amplification of the activity is beneficial to the detection of the ABR signal. However, much of the physiological myogenic activity is not the same at both inverting and non-inverting inputs and is thus not eliminated. The amount of

myogenic noise is also found to be high when testing infants. The noise levels can change dramatically between different states of awakeness. An additional noise source is internal noise from the instrumentation. This is only significant at the input stages of the preamplifier where noise is added to the electrode signal and is amplified by the gain. This noise source makes up a substantial proportion of the total effective input noise when testing sleeping infants.

2.3.4 Signal Filtering

Filtering is implemented to suppress frequencies which contain high levels of noise energy. This is another step in reducing the SNR. The filtering on the Bio-Logic Navigator, in use with this study, is digital. These operate by sampling the signal sequentially into a series of discrete numbers. The ideal filter would allow for the total suppression of some frequency components whilst leaving others unaltered. Real filters approximate this behaviour.

A bandpass filter passes frequencies between the upper and lower limits and rejects those outside. The lower limit refers to the high-pass filter where frequencies below the limit are suppressed and visa versa for the lower-pass filter. The sharpness with which the filter de-emphasizes the reject frequencies is given by its slope (also known as its roll-off, attenuation or rejection rate). This slope is expressed in dB/octave. Hence, a 24dB/octave slope means that the magnitude of a sound outside of the bandpass is reduced at a rate of 24dB for each doubling of frequency. The cut-off frequency is defined as the frequency where the power falls to half its peak value (the half power point). This point is 3dB below that of the peak and is commonly referred to as the 3dB down point.

The effectiveness of the filtering process is dependant on the overlap in frequency present between the signal and noise activity. If there is no overlap of signal and noise components then filtering can be very successful with high levels of noise being eliminated. However, the greater the overlap, the lesser the expected, and achievable, performance. The signal and noise spectra with the ABR overlap strongly and thus limit the effectiveness of the filtering process.

The overlapping nature of the activity also results in alteration of the signal. The filtering process can modify some signal frequency components and cause phase distortion of particular frequency components. It is known that low-pass filters will smooth out high frequency components and introduce a time lag. High-pass filters, on the other hand, will produce a time lead. This characteristic tends to affect the ABR more dramatically and can lead to suppressed peak amplitudes. However, with appropriate selection of both low- and high-pass filters, phase distortion of the ABR can be minimised. The electrical potentials of the ABR can have amplitudes less than 100 times that of surrounding electrical activity. Thus, ABRs will be undetectable unless a substantial amount of background activity is rejected. It is commonly believed that the main spectral components of waves I and II are distributed between 400 and 1000Hz; wave III between 100 and 900Hz, and waves IV-VI from 100 to 500Hz[11]. Kevanishvili and Aphonchenko[11] reported on adult hearing, they showed that the bandwidth setting for optimal resolution of the ABR is 150-1500Hz. It is also noted that for testing in harsh electrical environments, such as those found in a NICU, a bandwidth of 150-3000Hz is clinically acceptable.

2.3.5 Summation or Averaging

Following the filtering process the analogue signal is converted into a digital form. This approximates the value of activity at regular intervals into a discrete form. The summation or averaging of the digital waveform is a critical step in reducing the SNR. The identification of the ABR morphology from a single stimuli (an elementary record) is not possible. The elementary record contains activity from the signal and noise components. The basic principle in summation or averaging is that the signal response is synchronized with the stimulus and is deterministic. The noise component, however, is assumed to be random and will thus change over elementary records with time.

The Bio-Logic Navigator averages the elementary records, dividing the summed response by the number of records. This creates an ABR waveform that gradually converges into a response morphology. Both summation and averaging techniques provide the same benefits to the SNR. The quantification of the number of records that

are required for an appropriate response is hampered by statistical complexity and the fact that there is not a strong standardized measurement goal.

The specification of the number of sweeps to be taken for an ABR record is greatly influenced by the test environment, the likely muscle activity of the subject and the forehead electrode placement obtained. The testing for this current research is to be performed on preterm neonates, the ABR of these subjects generally being lower in amplitude than that of adults. Testing will be performed in the NICU resulting in high levels of background electrical activity. This results in the need to use a higher number of samples for averaging in order to obtain definable ABR peaks.

Edwards *et al.*[6] stipulated summing to 4000 sweeps for threshold testing, with higher intensities being tested to 2000 sweeps. Durieux-Smith *et al.*[7] also implemented this criteria, the high number of sweeps is used for threshold testing where the wave morphology may be less well defined. The test time is an important factor when examining neonates, a balance between test time and the desire for greater response definition must be found. The test protocol for this current study will be comprehensive resulting in a large number of test runs. The number of sweeps will, therefore, need to be limited due to time constraints.

2.3.6 Artifact Rejection

The occurrence of a large isolated artifact in an elementary record can have a dramatic affect on the ABR response even after averaging. Extremes of this type are commonly produced by myogenic activity. The artifact rejection process aims to eliminate these extreme records from the averaging process. Rejection occurs when the voltage at the analogue-digital converter (ADC) exceeds a limiting value. The Bio-Logic Navigator does not allow for alteration of this limiting value. It is therefore necessary to control the rejection level using the amplifier gain.

The level of artifact rejection can have significant affects on the quality of the averaged response. The major drawback of implementing a low rejection level is the time taken to acquire the given number of records. Practical data tends to contain periods of well-behaved low variance activity interspersed with bursts, sometimes lengthy, of high variance activity resulting from myogenic activity. Artifact rejection levels are thus set to

accept the majority of the well-behaved activity with all of the high variance activity being rejected. A rejection rate of approximately 10% is commonly used as a target value. However, when testing infants this value can be exceeded due to severe episodes of myogenic activity.

2.4 Test Protocol

2.4.1 Stimulus Rate

Numerous investigators[7, 8, 12] have studied the effect of different stimulus rates on the morphology of the ABR. In general, it has been reported that decreased amplitudes and increased latencies result from an increase in stimulus rate. The early waves (I-III) are more susceptible than the predominating wave V. However, the more immature the auditory system the greater the effect[12]. The physiological assumptions regarding the rate effect can be seen in Section 1.6.1.

When the screening of neonates is the most important factor, testing time can be significantly reduced by a faster stimulus rate. Klien *et al.*[13] found that for neonates with a PCA greater than 42 weeks, 92% of ABRs were present at 90 pulses per second (pps), all subjects having ABRs at 40pps (30dB nHL[c]). However, it was found that with increasing immaturity the success rate was dramatically reduced. Neonates with a PCA between 37 and 42 weeks were 71% successful at 90pps, with only a 50% success rate with preterms less than 37 weeks PCA. It is obvious from these findings that a stimulus rate of 90pps is unacceptable for detecting ABRs in preterm neonates.

Durieux-Smith *et al.*[8] studied neonates of varying PCA, testing for ABRs at 70dB nHL with rates of 11 and 61pps. They found that wave V latencies could be reduced by 0.6-1.3ms when reducing the stimulus rate from 61 to 11pps, the greatest latency reduction occurring in the younger subjects. It was also noted that testing with the higher stimulus rate at an intensity of 70dB nHL tended to wake the babies. A significant decrease in the amplitude of waves I and V was observed with increasing rate, wave III being relatively unaffected. Picton *et al.*[14] also studied neonates with stimulus rates of 11 and 61pps at 70dB nHL. They concluded that infant ABRs were significantly affected by rate. In general, an increased rate resulted in smaller amplitudes and longer

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[c] dB levels are relative to normal hearing level (nHL), unless otherwise stated.

latencies of all waves. The wave V amplitude was found to be less sensitive than earlier waves, waves I to IV decreasing rapidly beyond a rate of 10pps. It was found that between 50 and 90pps was the most efficient for recording of wave V. The morphology of the response was found to be simpler with the wave V peak being more prominent.

The selection of stimulus rate will strongly depend on the information required from the ABR. Durieux-Smith *et al.*[7] used a rate of 61pps at 30dB nHL to demonstrate normal peripheral auditory sensitivity, 11pps at 70dB nHL being used to assess neurological integrity of the auditory pathways of the brainstem. Hyde *et al.*[15] implemented a similar regime using 35pps to test for hearing thresholds with a rate of 21pps at 70dB nHL and above for otoneurologic assessment. Lina-Granade *et al.*[16] utilized stimulus rates of 20, 41.3 and 61.3pps with preterm infants from 32 to 39 weeks PCA. They studied the latency shifts between 61.3 and 20pps, and between 41.3 and 20pps. Significant latency shifts were observed for wave V with advancing conceptional age, no significant difference was found for wave I. They suggested that stimulus rates, used in this manner, can identify the area of the auditory system predominating the maturation, in this case changes were at a central level (beyond wave I).

2.4.2 Intensity - Its affect on Latency and Amplitude

The ABR is particularly sensitive to stimulus intensity, a decrease in intensity will result in an increased latency for all components. Below 60dB nHL, earlier waveforms tend to have reduced definition whilst the robust wave V decreases in amplitude and increases in latency. Commonly, the latency shift is in the order of 40μs/dB[16]. Changes in adult latency are non-linear but are consistent. However, infants display a greater variability in the change. It has been reported that the infant wave I exhibits two 'transitional zones', sudden changes in latency have been observed at 30-40 and 60-70dB nHL[17]. With increasing intensity, wave I normally decreases in latency faster than wave V, the interpeak latency (IPL) will therefore change as a function of intensity.

There have been differing interpretations of the infant wave V latency-intensity (L-I) function. In adults it has been used in differentiating cochlear and conductive pathology[18]. In infants, it has been suggested that adult L-I slopes are of a higher gradient[19] whilst others claim a lower gradient[4, 19]. Stockard *et al.*[19] reported steeper L-I

slopes for preterm over term newborns. Durieux-Smith *et al.*[8] found that infant waves III and V decrease significantly with increasing intensity, although there was no significant age by intensity interaction. This would suggest that the slope of the L-I function does not change with PCA. Gorga *et al.*[20] studied the L-I of the infant wave V. They found a small but systematic trend towards shorter latencies as age increased. They also reported that the shape of the L-I function remains virtually identical with age.

The amplitude of the infant ABR is also affected by changes in intensity, amplitudes reducing with decreasing intensity levels. A 'transition' of wave I amplitude occurs similar to the latency response. A doubling of wave I amplitude has also been reported between 60 and 70dB nHL[19]. Stockard *et al.*[21] reported that a third reduction in wave V amplitude (over a 50dB nHL reduction) can be matched by a ten-fold reduction in wave I. Picton *et al.*[14] found smaller amplitudes at lower intensities with wave V being less affected than earlier wave components, only wave V being recognizable at near threshold intensities. Durieux-Smith *et al.*[8] reported that wave III showed no significant change with intensity or age, with no interaction between variables. Wave V amplitude was found to reduce with decreasing intensity. Jiang[22] studied amplitude readings from neonates and infants. He found that all age groups showed similar amplitude-intensity (A-I) functions for waves I and V. Wave I exhibited a 'transitional' response at 60dB nHL, the variation of wave V being more gradual. A dramatic change in wave I amplitude (mean 57%) was seen when reducing intensity from 90 to 60dB nHL. Wave V showed a more moderate reduction (mean 30%), thus the V/I ratio more than doubled (mean 70%). In the moderate intensity range, the reduction in amplitude with decreasing intensity is only slightly more for wave I than V, the V/I ratio slowly increasing. Jiang[22] found the A-I function to have values between 0.024 and 0.042μV/dB over the different age groups. The V/I ratio increased with decreasing intensity by 0.01/dB, though not age related.

2.4.3 Threshold Testing

Threshold testing is an important strategy in assessing infant hearing, above 30dB nHL being considered by most as elevated for term infants[14]. Durieux-Smith *et al.*[8] found that preterm thresholds would generally be subject to elevation. They tested for

thresholds at 30dB nHL in an attempt to identify conductive hearing losses and, immaturity or pathological problems, for the brainstem and cochlea. They found that approximately 30% of the NICU infants passed the 30dB nHL threshold test at 32 weeks PCA. This figure rose to 70% and higher for infants born 33 weeks GA and later. However they indicated, during comparison with other reports, that bandpass filter settings (ie. 150-3000 Hz[23])[d] were a possible factor in the increased pass rate of early preterm infants. Hyde *et al.*[15] studied infants on a high risk hearing loss register, they found that commonly 8% of infants failed a 30dB nHL threshold criteria. Stockard and Westmoreland[24] stipulated a 40dB nHL threshold for infants of 28 to 34 weeks PCA, 30dB nHL for 35 to 38 weeks PCA, and found thresholds of less than 20dB nHL for full term infants.

2.5 Frequency Specificity

2.5.1 Frequency in the Cochlea

The use of specific frequency stimuli has been given little attention in previous research. The cochlea can analyze the frequency content of an auditory stimulus using the physical properties of the basilar membrane and cochlea fluids. The higher frequencies will vibrate the basal[e] regions of the basilar membranes whilst lower frequencies will vibrate all regions of the basilar membranes (most vibration occurring in the apical[f] regions). A 'place' coding on the basilar membrane will result from this travelling wave.

Examining the physical properties, the travelling wave has a finite velocity, decreasing as it moves along the basilar membrane. In an adult human, the wave takes approximately 5ms to travel from the base to the apex of the cochlea[1]. It is, therefore, correct to assume that low-frequency sounds are initiated later than high-frequency sounds. The travelling wave is also asymmetric, high frequencies activating only high-frequency regions of the basilar membrane, whereas low frequencies activate both high- and low-frequency regions (basal and apical). With the decreasing velocity of the

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[d] These filter settings may provide more reduction to the interference signals of the NICU environment.
[e] Refers to the base region of the basilar membrane.
[f] Refers to regions of basilar membrane nearer to the apex.

travelling wave, the extent of the basilar membrane specifically activated by the particular frequency increases as the wave moves from base to apex. The mapping of frequency to distance is approximately logarithmic. Frequency specific stimuli can be generated by either modified clicks or tone bursts (pips). These sounds can be presented unmasked, masked with high-pass noise, or masked with pure tones (or bandpass noise). With the equipment being used for this study, a tone burst without masking has been identified as providing an appropriate frequency specific stimulus.

2.5.2 Unmasked Tone ABRs

The commonly used, standard broadband click stimulus has a frequency range of 2-4kHz. It would follow that brief high-frequency tone bursts might produce brainstem responses similar to those elicited by standard click stimuli. Low-frequency tones (<2kHz), however, would produce traces with significant degradation due to a reduction in the synchronization nerve fibre firing[28]. More recently, with better quality instrumentation, identifiable peaks have been found with low frequencies. Davis and Hirst[25] identified the vertex-negativity following wave V as being the most prominent component of the response to low-frequency tones. This has since been named the "slow negative wave at 10ms" or SN10.

Brief tones differ from click stimuli in two characteristic ways; rise time and frequency content. Tones can be constructed in two ways; rise times of a particular number of cycles and those having a fixed period of time regardless of frequency. Various set-ups have been recommended, including the "2-1-2" tone, having rise and fall times of 2 cycles and a plateau duration of 1 cycle. Stapells and Picton [26] suggested the implementation of a 5ms rise time regardless of frequency.

For tonal stimuli, the amplitude of the response decreases with increasing rise time, this becoming more accentuated over 5ms. Wave V latency displays complex variations with rise time, frequency and intensity of the tone. Hecox and Deegan[27] used high-pass noise techniques to investigate the affect of rise time on wave V latencies. They found a change from 2 to 5ms in rise time could increase wave V latency by 0.5ms.

Frequency variation of the tone has significant affects on the morphology of the response. Wave V latency is longer with low-frequency tones, this increase reflects the

additional time for the travelling wave to reach the low-frequency regions of the basilar membrane near the apex of the cochlea. This latency difference is strongly dependant on the intensity of the tone. For example, at 90dB nHL wave V latency is increased by 1ms from 500 to 4000Hz, at 40dB nHL the difference is approximately 3ms[26]. Care must be taken not to use too high an intensity as this can cause spread of energy in the tone and travelling wave. Thus high-intensity, low-frequency tones can be mediated by the high-frequency region of the cochlea, a lower intensity would reduce the amount of energy spread.

Abdala and Folsom[28] studied frequency contribution to ABRs in infants using clicks in notched noise. Target centre frequencies varying from 500 to 8000Hz were examined. Infants used in the study were between 11 to 13 weeks old, born at term with normal hearing. They found the low-frequency dominated the infant response with adult like wave V latencies occurring earliest in the low frequency region. The frequency specific traces were compared with traces using broadband click stimuli. They concluded that traces that were stimulated with frequencies between 2 and 8kHz had similar morphology and latencies to the broadband click (waves I, III and V being identifiable). At frequencies of 500 to 1500Hz, early waves (I and III) became less noticeable, wave I usually not being present. Wave V was found to broaden substantially due to the low-frequency contribution, but remained robust. The latency of low-frequency responses was found to be higher due to additional travel time on the basilar membrane, the latency shift being greater for wave V than wave I. They also found that wave V latency increased gradually as the notched-noise centre frequency was varied from 8000 to 500Hz. They concluded that, the high levels of latency elevation at these low frequencies suggest this frequency region has little importance on the attributes of the standard unmasked click response. Latency values and morphology for the 4 to 6kHz region were most similar to the unmasked responses. Thus, standard click traces are most influenced by responses in this region.

2.6 Morphology Descriptors

ABR peak components are commonly labelled using Roman numerals (I-VII), as described by Jewett and Williston[29]. The interpretation of these peaks are generally based on, latency (in ms) of individual peaks, latency differences between peaks (interpeak latency), peak amplitude (in μV), amplitude ratio's (ie. I-V), and general wave morphology.

The absolute latency of a wave is measured from the onset of the acoustic stimulus to the peak of the averaged response. Currently, no standard exists as to the exact point on the peak for the measurement of latency. A point representing the beginning of the downward slope of the peak complex could be identified. Also used is the steepest gradient of the downward slope. This convention is advantageous when multiple peaks are present. The latency of wave V has particular importance due to its robust nature and its reliable variation with intensity. Amplitude measurements still have limited success as an auditory diagnostic tool, values tend to be more variable and not normally distributed. They also tend to be difficult to replicate, influenced by minor alterations in recording technique and susceptible to interference from myogenic activity[8] and noise levels. Amplitudes can be measured from a base-line to a peak (for absolute values), from peak to following trough, or by peak to peak ratio's.

Edwards *et al.*[6] and Durieux-Smith *et al.*[8] identified criteria for latency and amplitude measurements. Wave I is the first positive peak after 1.3ms, where several peaks occur within 0.7ms the middle latency among peaks of similar amplitude is identified. Wave III is the first positive peak after 3ms with the same conditions as for wave I. Wave V is identified as the first positive peak after 5ms, it being marked just before the rapid negative deflection. Amplitude measurements relative to a computer generated base-line can create misleading results. They suggested that for waves I and III the amplitude is identified as the tip of the positive peak to the maximum negative trough within 2ms. Wave V has similar criteria with a maximum trough within 3ms. Comparison of results from different studies is still difficult, this is largely due to the varying styles of wave morphology description criterion. Peaks are identified at varying points and multiple peak complexes are assessed differently.

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[8] Originating from muscle tissue.

2.7 The Infant ABR

2.7.1 Detectability of ABRs in Preterm Neonates

There is still a lack of normative data on the ABR characteristics in the preterm infant[30]. It is not completely understood how preterm birth affects the brain maturation process[9]. Mercuri *et al.*[31] found that myelination of the Central Nervous System (CNS) does not occur until 40 weeks PCA. However, many studies have found detectable ABRs during this maturation time. Krumholz *et al.*[32] studied preterms at 65dB nHL from 25 weeks PCA. They found that between 25 and 27 weeks PCA none of the three infants tested produced detectable ABRs. Between 27 and 29 weeks PCA, three out of five infants produced traces. Beyond 30 weeks PCA, reproducible traces were obtained from nearly 100% of infants. At this age, waves I, III and V were found to be more stable than waves II and IV. They concluded that ABRs approach adult characteristics by 38 to 43 weeks PCA, approximately 60 to 80% of these subjects also displayed distinct waves II and IV. Mercuri *et al.*[31] found waves I, III and V were possible to obtain at 25 weeks PCA at 70 dB nHL. The maximum change of wave morphology was found to exist between 29 and 36 weeks PCA. Downs[33], however, criticized the use of ABRs as a screening tool in the NICU because of the lack of reliability and validity, inadequate normative data, and poorly standardized procedures.

2.7.2 Testing in the Neonatal Intensive Care Unit (NICU)

The NICU is a hostile environment for the measurement of electrical EPs. However, it is possible to obtain good results with quality instrumentation and a flexible test protocol[32]. There are numerous sources of potential electrical interference; fluorescent lights, mechanical ventilators and various monitoring devices. Surrounding rooms may also provide a source of interference due to the lack of electrical shielding present in a NICU, mains 50Hz line noise will also be present. It could be beneficial to use a power outlet not being used for other equipment, to use a stimulus rate not divisible into 50Hz (ie. 13, 61, etc.) and to use a minimum of 2000 sweeps. The high-pass filter may need to be altered to suit a particular environment, 150 to 300Hz to eliminate 50Hz noise contamination.

Thomas[34] reported that a typical NICU environment provides low frequency sounds of constant high intensities, commonly 70 to 80dB nHL. It is known that prolonged or loud sounds can damage the hearing of an infant in that frequency range. Incubators have been identified as producing excess amounts of noise, incubated infants being more susceptible to hearing losses in the incubator noise frequency. Nzama *et al.*[35] studied noise levels in a NICU and found a considerable level of noise (>60dB nHL) which persists throughout the day and night. They classified noise as originating from either environmental, equipment related, personnel or patient related sources.

2.8 Parameter and Protocol Selection

The following parameters were identified as being suitable for the subjects and test environment present in this study. Filtering will be between 100-3000Hz, tests being averaged over 2000 sweeps. The high forehead will be used for the positive electrode site, the mastoid placement being favoured over the earlobe location for the negative site. A note will be made of the positive electrode location, younger subjects sometimes requiring a lower forehead placement. Impedances will be kept below 5kΩ with well balanced values.

Figure 2.2 shows the test protocol. Due to the broad range of the test protocol only one ear will be examined. The ear will be selected during the first test session, the selection will be random except in cases where access to a particular ear is easier. A screening procedure will be performed before each test session to check for elevated latency values in the test ear (compared to the non-test ear). Readings will be taken (13pps) starting at 80dB nHL decreasing in 10dB nHL steps (to a minimum of 10dB nHL) to develop L-I and A-I functions. Definable wave V morphology will be taken as the criterion for threshold. Stimulus rates of 13, 37 and 61pps will be used to identify the area of the auditory system predominating the maturation process. The higher stimulus rates will only be tested at 60dB nHL. These will be used to assess the rate effect. Frequency specific tone bursts of 500, 1000 and 4000Hz will be used to identify maturation in different frequency ranges. Tone bursts of the "2-1-2" type will be implemented, having rise and fall times of 2 cycles and a plateau duration of 1 cycle. Two runs will be performed to assess repeatability and accuracy for rate and frequency.

Due to test time limitations, the L-I relationship will be used to check data recorded at 13pps. This will enable a lesser number of tests to be performed.

Wave morphology will be marked using the various techniques available; absolute latency, IPLs, absolute and relative amplitudes, and amplitude ratio's. It is anticipated that ABRs will be detectable in infants from 28 weeks PCA and younger at intensities of 80dB nHL. However, the high levels of interference in the NICU may delay detectability.

2.8.1 Validation of Collection Parameters and Test Protocol

Initial testing was carried out on newborns undergoing standard hearing assessment in the Newborn Hearing Unit. The testing, normally undertaken on a Medalec portable ABR instrument, was performed on the Bio-Logic Navigator ABR machine. A total of nine babies were tested to validate the working parameters of the machine and to check the suitability of the test protocol.

Testing was commenced with term infants. It was found that reduction of the signal gain (from 300,000 to 200,000) was necessary to control interference. This interference led to an increased number of rejected artifacts. These artifacts are produced when the response exceeds prescribed limits. This process is useful for eliminating background interference, as well as electrical activity caused by body movement. Further testing enabled identification of wave V at both 60 and 40dB nHL. There was also some evidence of waves I and III. The signal gain was once more reduced to 200,000, this lowered artifact rejection. Further testing concentrating on gain values showed that responses could be achieved with the maximum gain value (300,000)[h] in suitable conditions. It was concluded that the gain might have to be reduced (240,000 or 200,000) for restless subjects and for testing in the NICU.

This initial testing was extended to preterm infants in order to establish the levels of response possible with the equipment available and the particular environment. The aim of these tests was to record a set of responses at different intensity levels (80, 60, 40dB nHL). Clear waves were produced at 80 and 60dB nHL, waves I, III and V being present. The 40dB nHL showed only a poorly defined wave V. Further preterm subjects

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[h] With higher gain values the number of rejected artifacts is higher. This is not a problem to the accuracy of the recordings but does increase test time.

were tested with the full protocol, tests performed on infants (in the NICU) of 32 to 36 weeks PCA proved successful.

2.9 Conclusion

The collection parameters for the Bio-Logic Navigator machine in use for this study were established. Appropriate signal filtering settings were identified for optimum performance of the Bio-Logic Navigator in the NICU environment. The number of sweeps per test was established to compromise wave definition and reliability with test time limitations. It is envisaged that each test run (with a low stimulus rate of 13pps) will take 3 minutes. This time could be prolonged with restless subjects, a greater number of rejected artifacts being produced. A suitable electrode montage and application procedure was established through the review and discussions with clinical staff. A routinely used electrode location procedure was accepted, this will provide consistency to electrode application.

A comprehensive test protocol was established. The test session time available permits the testing of one ear only. However, this strategy allows for a broader range of parameters to be investigated. These will include; a full range of intensities (allowing observation of the L-I relationship) and various stimulus rates (allowing observation of the rate effect). The initial testing with specific frequency stimuli was found to produce poorly defined data with a lack of peaks for marking. This area of investigation will not be continued. The protocol will provide the maximum amount of useful information for assessment of the maturational characteristics of the auditory system.

Various wave marking criteria were reviewed. Initial experimentation allowed assessment of the most appropriate criteria for use with the data obtained. Strict criteria was established to ensure consistency and reliability of numerical data. Waves I and III will be marked at the highest peak or at the centre peak if a complex of peaks is present. The wave V peak was often poorly formed, multiple peak complexes were generally observed. The most reliable marking criteria for wave V was found to be the steepest point on the trailing slope of the peak complex. Amplitude data was found to be unreliable and extremely variable, especially for the wave V complex. It was concluded

that the data obtained was not suitable for an amplitude analysis. Amplitude parameters will not, therefore, not be discussed further.

The review on the detectability of ABRs in the preterm infant has suggested that from 30 weeks PCA a high level of detectability is expected (≈100%). The collection of ABR data from infants before 30 weeks PCA will be attempted. A reduction in wave definition and quality is probable. The collection parameters selected will aid in the acquisition of early data from neonates in the NICU. It is expected that a high percentage of infants tested beyond 30 weeks PCA will display ABR waves I, III and V. Other waves (II and IV[i]) tend to be poorly defined and will not be utilized.

[i] Wave IV is often included in the wave V complex.

Figure 2.1 International 10-20 system for electrode placement

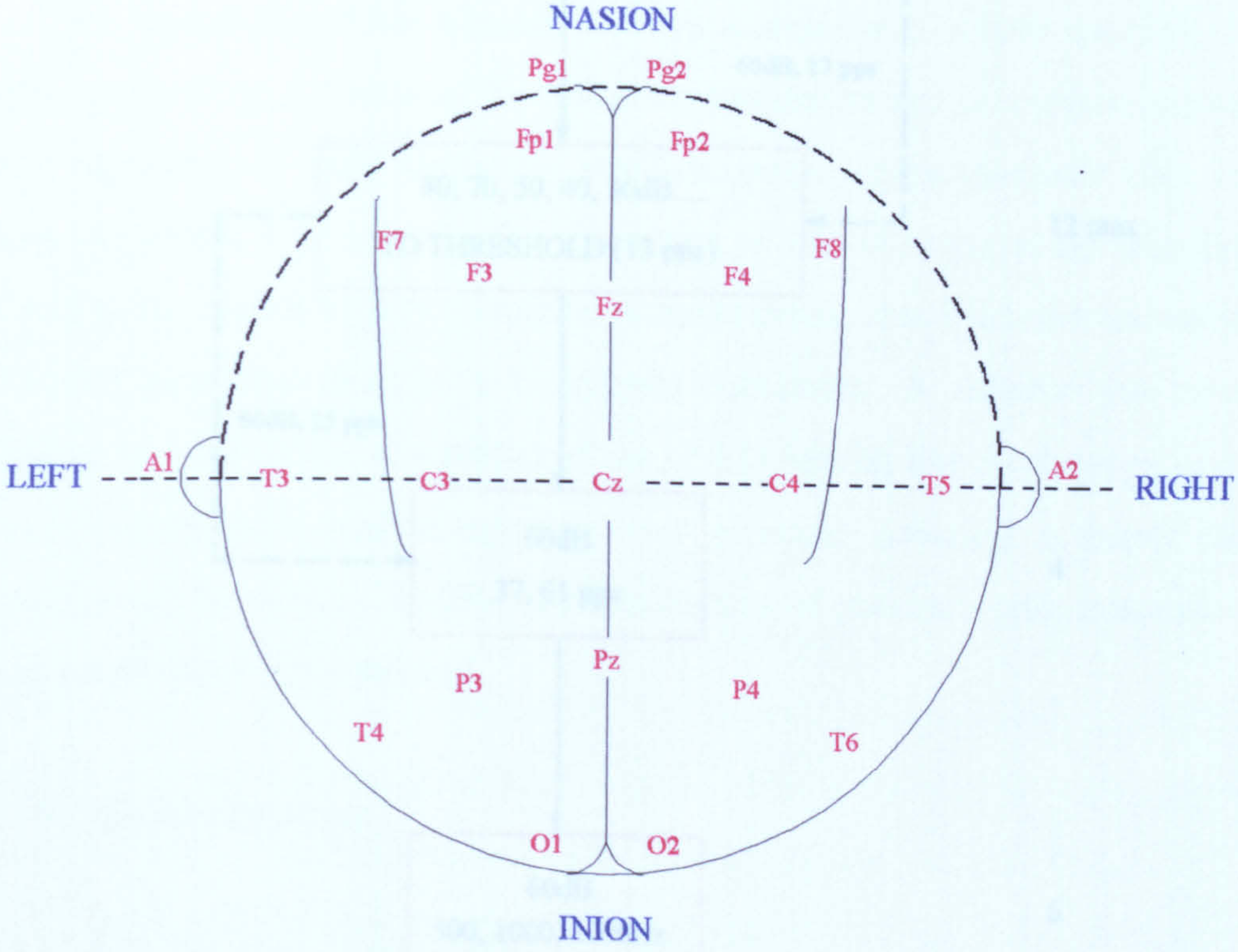
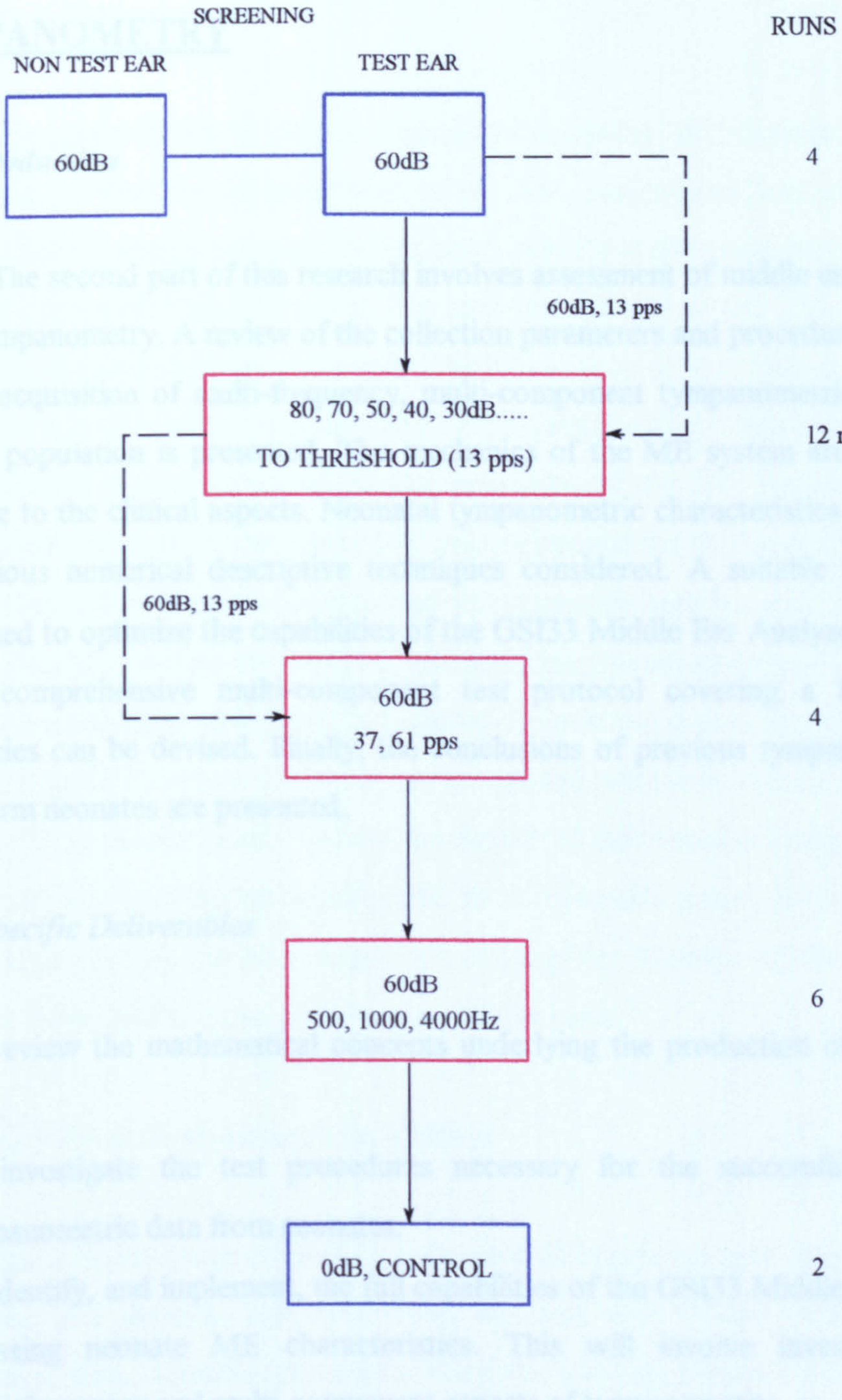


Figure 2.2 Test Protocol



CHAPTER THREE

TYMPANOMETRY

3.1 Introduction

The second part of this research involves assessment of middle ear (ME) function using tympanometry. A review of the collection parameters and procedures necessary for reliable acquisition of multi-frequency, multi-component tympanometric data from the neonate population is presented. The mechanics of the ME system are discussed with reference to the clinical aspects. Neonatal tympanometric characteristics are investigated and various numerical descriptive techniques considered. A suitable test protocol is established to optimize the capabilities of the GSI33 Middle Ear Analyzer. It is intended that a comprehensive multi-component test protocol covering a broad range of frequencies can be devised. Finally, the conclusions of previous tympanometric studies on preterm neonates are presented.

3.1.1 Specific Deliverables

- To review the mathematical concepts underlying the production of tympanometric data.
- To investigate the test procedures necessary for the successful acquisition of tympanometric data from neonates.
- To identify, and implement, the full capabilities of the GSI33 Middle Ear Analyzer in assessing neonate ME characteristics. This will involve investigation of the multi-frequency and multi-component aspects of tympanometry.
- To observe tympanogram characteristics found in the neonatal population.
- To establish the developmental changes occurring in tympanometric data during the preterm period.

- To investigate the various descriptors of tympanometric data, identifying useful techniques for the data expected.

3.2 What is Tympanometry?

Tympanometry is an objective means of analyzing ME function, it measures the compliance or freedom of movement of the ME components and can estimate ME pressure. Sound energy entering the ear canal stimulates the tympanic membrane (TM) setting the ossicular chain in motion. The malleus, attached to the TM, sets the incus in motion which in turn triggers the stapes. The stapes is set in the opening of the inner ear known as the oval window. The mobility of the ossicular chain dictates to a great extent the quantity of sound perceived by the individual. If the ME system is stiffened or disturbed for any reason, the amount of energy transmitted to the oval window will be altered. The sound is then conducted by the stapes through the oval window into the inner ear (cochlea) where the energy is translated into meaningful signals for the brain.

The eustachian tube plays an important role in the discussion of ME function. It provides a route by which ME pressure is equated with atmospheric pressure, or the pressure within the ear canal. Without the benefit of the eustachian tube to equalize ME pressure the eardrum would be subjected to considerable stress. As a diagnostic tool, tympanometry is an objective means of assessing the function of the ossicular chain, eustachian tube, TM and the interrelation of these parts.

3.3 Basic Principles of Acoustic Immittance

Acoustic immittance refers to both acoustic impedance and admittance. Acoustic admittance is a general term quantifying the ease with which sound energy travels through a system. Acoustic impedance being the total opposition to the flow of sound energy. These two quantities are reciprocal. A system with a high acoustic impedance (opposition) to sound energy transfer will have a low acoustic admittance.

When sound reaches the ear canal, sound pressure is applied to the eardrum. This pressure will set the eardrum and the middle and inner ear systems in motion, transferring the energy. Measuring the energy flow taking place at the lateral surface of the cochlea

can help describe the energy transfer process of air particles in the ear canal to electromechanical activity within the cochlea. The ME system, however, is not a perfect transducer of sound energy. Not all the energy presented at the TM will flow through the ME system. The opposition to transfer in this system is known as the acoustic impedance (Z_a), measured in acoustic ohms. Its reciprocal is the acoustic admittance (Y_a, units are mhos), the ease with which energy is transferred from the eardrum into movements of the ossicular chain and cochlear fluid.

3.3.1 Plane of Measurement

Whether acoustic immittance measurements are described in terms of impedance or admittance, the method of measurement is the same. Measurements are achieved by presenting an acoustic signal to the ear and observing the reflected sound energy. A probe signal (tone) in the ear canal will cause a sound pressure level (SPL) which can be used as an indirect index of immittance. The SPL will be proportional to the immittance, the greater the SPL measured at the probe tip (reflected energy) the lower the equivalent admittance of the ME.

The probe is placed in the ear canal, an hermetic seal is in place. The plane of measurement of the input acoustic immittance of the auditory system is at the probe tip. This measurement of total immittance at the probe tip, at ambient or atmospheric pressure, includes contributions from the ear canal, TM and the entire ME system. The desired point of measurement is the TM. Therefore, to define the input acoustic immittance of the ME system at the TM, elimination or subtraction of the ear canal contribution is necessary. The ear canal contribution is determined by taking immittance readings with substantial canal pressures in place[a]. The impedance at the probe tip increases as the pressure is changed from ambient. The pressure change in the canal has the effect of stiffening the ME and hence the ME system[1]. Admittance will reduce to a minimum (maximum impedance) with sufficient pressure. At this point, the immittance represents the proportion of the total immittance occurring due to the volume of air in the canal. This canal immittance can then be used as a baseline value for other readings. The corrected values represent the input immittance of the ME system at the plane of the

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[a] Positive and negative extremes can be used, the choice will be discussed later in this chapter.

TM. This enables the standardization of immittance testing, independent of the ear canal volume and probe tip location. These measurements are termed 'compensated' static acoustic immittance, probe tip values being 'plane' measures.

3.3.2 Transmission of Energy within the Ear

Energy transfer is present when the TM and ME structures are set in motion. As in all mechanical systems, transfer does not happen instantaneously with applied force. The different mechanical structures of the ME system react in a variety of ways, immittance is a complex relationship between force (pressure) and velocity (movement of the various structures). The acoustic impedance (Z_a) is the vector sum of the magnitudes of the divergent forces opposing the transfer of energy. It is given by the complex ratio of a sound pressure to a volume velocity at a given surface. The acoustic admittance (Y_a) is given by the inverse of this ratio. A similar complex ratio may be used to describe the result of forces that act at different times rather than divergent directions. The combination of two forces that are out-of-phase can be described by a phasor or vector sum, this phasor always arising from the origin. The phasor sum, like the vector sum, is the net magnitude and time difference that results when the two forces are combined.

The acoustic immittance found at the TM is controlled by many parameters; the mass of the three ME ossicles, the stiffness of the ossicular ligaments and muscles, the stiffness of the TM and round window membrane, the stiffness of the air contained in the ME, the mass and friction that result from the air movement within the ME, and the total immittance of the cochlea at the oval window[2]. The acoustic immittance is, therefore, controlled by a variety of masses, stiffness and frictions. In order to simplify matters, there are basically two main components involved in the transfer of sound energy. These components are the in-phase component (ie. simultaneous with applied force) and the out-of-phase components (ie. those occurring after the force). The in-phase component is equivalent to the heat produced by friction when a force acts. Acoustic resistance (R_a) is the dissipation of sound pressure or acoustic energy. Acoustic conductance (G_a) is the inverse. The acoustic resistance quantity is independent of frequency[b]. Acoustic reactance (X_a), and acoustic susceptance (B_a), are the out-of-phase component

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[b] This is not always the case for measurements of complex systems such as the ear.

controlling acoustic energy transfer. These quantities are out-of-phase with the applied force. These components store energy in stiffness or mass elements, a velocity is subsequently produced.

In order to fully understand the mechanics of the ME system, the mathematical concepts behind acoustic immittance should be considered. The basic mathematical principles of tympanometry are presented in Appendix B.

3.3.3 Ear Canal Correction - Plane of Measurement

As previously explained, compensated acoustic immittance values are required for analysis because of the variations in ear canal size and the specific immittance properties of the air in the ear canal cavity. The ear canal admittance (Y_{ec}) must, therefore, be found from the immittance data and subtracted from the overall admittance (Y_a). This will give the admittance in the plane of the TM (Y_{tm}). As previously mentioned, Y_{ec} is estimated from immittance data at the extreme positive or negative values. The GSI33 machine uses +200decaPascals (daPa) for the ear canal correction procedure, this can be activated by selecting the 'Baseline on' command. The tympanogram then has a positive pressure tail value of zero[c]. It has been established from initial testing, supported by Wiley *et al.*[3], that the negative pressure tail will then have a negative immittance value. This would suggest an over estimation of ear canal immittance. Shanks and Lilly[1] reported that compensated admittance at peak pressure was 19% higher when volume estimation was carried out at -400 as opposed to +200daPa. Additionally, they reported that ear canal volume was 9% higher when estimated using the susceptance versus the admittance tympanogram. They concluded that volume is best estimated from the minimum susceptance value, usually occurring around -400daPa.

The GSI33 instrument is manufactured to estimate ear canal volume using +200daPa pressurization. For this study, compensation will be calculated using an extreme negative value (-390daPa, measurement at exactly -400daPa not always

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[c] A tail value refers to extreme pressure values (either -400 or +200daPa for this study). The 'baseline' option on the GSI33 uses +200daPa as the reference immittance value and thus will have zero immittance at +200daPa. Alternatively, it is possible to record absolute values and to compensate data retrospectively to the negative tail (-400daPa). This option is further discussed in Chapter Six.

recorded due to equipment set-up). The GSI33 will be operated with the 'Baseline' option disengaged, compensation being performed post-test.

Corrected and uncorrected admittance tympanograms are parallel in form due to their linear relationship (see equation (24) in Appendix B). There is an important assumption implicit to the use of the compensation equations in Appendix B. This assumption is essential for the correction of both impedance and admittance measurements. It is necessary to assume that the phase angle[d] of the ear canal impedance is identical to the phase angle of the ME impedance. This assumption is reasonably accurate for normal adult ears at low probe tone frequencies where the ear canal and ME are essentially compliant (stiffness) elements. This is not the case for many abnormal ears, for normal ears tested at higher probe tone frequencies, and for normal neonate ears even at low probe tone frequencies. In these cases, separate measurement of individual impedance components (resistance and reactance) or admittance components (conductance and susceptance) is required. The individual components can be corrected for ear canal volume separately, then impedance or admittance data formulated. For this reason, equipment used in the study will measure the real and imaginary components of admittance (conductance and susceptance) separately.

3.4 The Tympanogram

3.4.1 Basic Test Procedure for Tympanogram Measurement

The probe is positioned at the opening of the ear canal, a hermetic seal is essential for accurate recordings. Once the seal is in place a known amount of sound energy is introduced, a measurement of the energy not absorbed by the system is recorded. The amount of sound energy transmitted is equal to the amount of sound energy introduced minus the energy that returns to the probe microphone.

The probe introduces a pressure of approximately +200daPa[e] into the ear canal (1.02mm H_20 = 1.00daPa). This positive pressure forces the TM inward and allows the approximate ear canal volume to be measured (with 'Baseline On'). This volume

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[d] Refers to the assumption that the phase angle of the sound energy is the same at the probe tip as that in the plane of the TM.

[e] For a positive to negative sweep direction, tests can also be performed in the opposite direction. Sweep direction will be discussed later in this chapter.

provides the baseline from which the tympanogram is drawn (if using inbuilt compensation software). The pressure is then automatically varied in the negative direction, the admittance of the system being constantly monitored. The pressure continues in the negative direction until a prescribed limit (-400 and -600daPa are commonly used). Thus, a tympanogram displays the admittance whilst the pressure in the ear canal is varied in a controlled manner. A maximum value is observed when the canal pressure is equal to that within the ME[f]. In an ear with a normally functioning eustachian tube the peak admittance will occur at atmospheric pressure (ie. 0daPa). The peak admittance can be reduced in amplitude by stiffening of the TM as happens with an otosclerotic condition. However, the admittance range for normal ME operation is fairly broad. Therefore, care must be taken when attempting to diagnose particular conditions.

An increase in peak amplitude can be caused by ossicular discontinuity or a continued ME effusion resulting in thinning of the TM. However, tympanometric results on their own must be viewed carefully as more than one condition can exist simultaneously. A shallow flat curve with a very negative maximum admittance in adults suggests two or more conditions at work. However, in infants it is an indicator of otitis media with effusion (OME). A flat tympanogram can identify extreme stiffness or perforation of the TM. With a perforated TM, it is not possible to create a pressure differential across the membrane. The ear canal volume will be greatly increased. This also applies with an artificial perforation (ie. grommets).

3.4.2 Multi-frequency Testing

For the majority of clinical immittance testing a single probe tone of low frequency (approximately 226Hz) has been utilized. This is generally because of the lack of multi-frequency equipment available. Common ME diseases (ie. otitis media, eustachian tube dysfunction, otosclerosis) can be detected and adequately described using a low frequency. Over many years, investigations have found that the clinical information obtained with multi-frequency tympanometric recording provides more insight into ME assessment and pathology than standard single probe frequency data[7]. New immittance systems are capable of multiple probe tone frequencies (200-2000Hz).

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[f] ME pressure estimation using this characteristic is very variable and unreliable.

However, most clinical data is concerned with single low frequency (220 or 226Hz) testing, sometimes with one additional high frequency (660 or 678Hz). The tympanograms generated with high or multiple frequencies tend to be more complex and thus more difficult to interpret. Van Camp *et al.*[6] presented the case for using high-frequency tympanograms. They recommended either a 600Hz, or preferably 678Hz (an integral multiple of 226Hz) probe tone. The reasons put forward for using a higher probe tone frequency are; low-impedance abnormalities produce unique tympanogram shapes only for the higher frequencies, contralateral reflex[8] measurements are possible in neonates (226Hz only useful in adults), and ipsilateral reflexes are contaminated by artifacts at 226Hz (not at 660Hz).

3.4.3 Multi-component Testing

Clinical testing is generally undertaken on simple screening tympanometers which can only record basic admittance measures. Admittance has two components; susceptance and conductance. With more complex measuring instruments, these components can be measured individually. This can provide information on the status of the individual mechanical processes within the ME system. Mass susceptance (B_m) is the ease with which energy flows into a mass, stiffness susceptance (B_s) being the ease with which energy flows into a spring element. Conductance (G) is a measure of the ease with which energy flows into a resistive (frictional) element. The information obtained by this more detailed recording technique provides significantly more information than simple admittance (Y) measurements alone. It also allows for the calculation of compensated values in neonates at higher frequencies. Compensation must be performed on the individual components as previously stipulated.

[8] The acoustic reflex is not being considered in this thesis. However, some test results for the ipsilateral acoustic reflex obtained during testing for this study are included for reference in Appendix E.

3.5 Tympanogram Descriptors

3.5.1 Descriptors of Single Peaked Tympanograms

Researchers have put forward classification systems for single-component, low probe tone frequency (226Hz) tympanograms[4, 5]. Low frequency tympanograms tend to be single peaked. Jerger's[4] system has become popular because of its simplicity. He classified tympanograms as being of three types; A, B or C (fig. 3.1). Type A is considered to be a normal tympanogram, it has a peak (maximum admittance) near normal atmospheric pressure, within the 0 to -100daPa range. A type B tympanogram has no obvious peak of maximum admittance. Admittance changes with pressure variation are very small, the curve can be virtually flat. This type is usually associated with fluid in the ME space. Type C does have a distinct peak, however, it occurs at a pressure exceeding the -100daPa value. This type of tympanogram usually reflects dysfunction of the eustachian tube, this can be a precursor to serious otitis media (SOM). The type C trace can also evolve into a type B with no peak. Other classifications also exist; type A_s (shallow type A) tympanograms reflect reduced mobility of the ossicular chain, type A_d (excessively deep type A) shows a highly compliant ME system produced by a disrupted ossicular chain or a scarred TM. The use of higher probe tone frequencies (678, 1000Hz) will change the classification criterion, W-shaped and undulating tympanograms being observed. The clinical use of tympanometry is based on these simplified pattern classifications of basic admittance data (susceptance and conductance is not presently used).

3.5.2 Descriptors of Multi-peaked Tympanograms

It has been established that most low frequency (226Hz) tympanograms are bell shaped with a single maximum admittance peak. The complexity of multi-component, multi-frequency tympanograms varies depending upon the number of peaks and troughs present for each component at each frequency. Peaks of maximum and minimum admittance are termed extrema. These tympanograms were first investigated by Liden[9], he named them 'W' tympanograms due to the often encountered inverted 'W' shape of the admittance plot. Mathematical analysis was carried out by Vanhuyse *et al.*[10], they

established that normal ears could produce multi-peaked tympanograms previously thought to be the result of ME pathologies.

The Vanhuyse *et al.*[10] model is reproduced in Figure 3.2. It shows the shapes of susceptance and conductance tympanograms based on the relationship between the resistance and reactance data. All the resistance tympanograms are identified as decreasing with increasing positive pressure. The reactance tympanograms are inverted 'V' shapes. These 'V' shapes shift towards more positive immittance values which are indicative of less stiffness dominated systems. The least complex normal pattern has one extrema for susceptance (B), one for conductance (G), and one for admittance (Y) (a 1B1G1Y classification). The majority of normal ears, at low frequency, will have this bell-shaped pattern for each component. However, other normal patterns do exist. The 1B1G pattern is found in normal adult ears at low frequencies and in 75% of adult ears at 678Hz. This pattern occurs when the stiffness reactance is larger than the resistance for all pressures. The 3B1G tympanogram occurs when the stiffness reactance is smaller than resistance near the peak, reactance being larger than resistance at more extreme pressures. This results in a notched susceptance tympanogram (three extrema) and a single peaked conductance tympanogram. The admittance tympanogram also remains single peaked, although a notched admittance (3Y) pattern is sometimes found[11]. The 3B1G pattern is displayed in 20% of normal ears for high frequency probe tones[3]. Notching in both susceptance and conductance (3B3G) tympanograms occurs when reactance is positive (this constitutes a mass controlled system), the reactance is smaller than resistance near peak and greater in absolute value at extreme pressures. The admittance tympanogram will also notch when reactance becomes positive (3B3G3Y). Creten *et al.*[8] found this pattern in approximately 4.5% of a normal adult population. A ME producing this type of tympanogram is mass controlled. In 5% of normal adult ears, a 5B3G tympanogram is observed. This happens when the reactance is positive and greater than resistance near peak, this results in a broadly notched admittance tympanogram.

Van Camp *et al.*[12] reported additional criteria to confirm a notched tympanogram as normal (in the adult). Firstly, notched tympanograms should not occur at low frequencies, even in neonates. The number of extrema must not exceed five for susceptance and three for conductance. Also, the pressure difference between outermost

conductance maxima should not exceed the susceptance maxima pressure difference. Finally, the pressure difference between outermost maxima must not exceed 75daPa for 3B3G tympanograms and 100daPa for 5B3G tympanograms. The suggestion that neonate tympanograms should not be notched at low frequency will be discussed in the next section.

3.6 Neonate Multi-frequency Tympanometry

3.6.1 Clinical use of Tympanometry in the Neonate

Immittance measurements began to be used in clinical audiometric assessment in the 1970's[4]. Conventional measures of admittance require a compensated value, the total admittance minus the admittance caused by the ear canal volume. As expected, ear canal volumes vary widely with age, neonates having ear canal volumes of $<0.50cm^3$. As well as volume changes, Holte *et al.*[13] reported that the external canal wall characteristics will also change in the first few months of life. Ear diameters can, therefore, be changed with pneumatic stimulation. This (possible) change is reduced with age. Their research concluded that before 2 months of age tympanograms would be affected by canal movement. This method of testing, thus, being an inappropriate tool. However, other studies (mentioned later in this chapter) suggest that reliable tympanograms can be achieved in neonates.

3.6.2 Multi-frequency, Multi-component Tympanometry in the Neonate

There is currently a lack of normative tympanometric data for the preterm population. Holte *et al.*[13] studied multi-frequency tympanograms in the first four months of life. They performed two component admittance tympanometry on 23 healthy term newborns in the 226 to 1000Hz range (226, 355, 450, 560, 630, 710, 900Hz). Their initial tests were carried out at 2 days of age; uncompensated susceptance, conductance, reactance and resistance tympanograms were recorded. They found that at this age the resistance and reactance tympanograms were irregular in morphology with high resistance and reactance values compared to adult data. The resistance and reactance tympanograms at 226Hz (between -200 and +200daPa) were found to have similar

morphology to adult patterns, but the minimum reactance (near 0daPa) was identified to be less than resistance. In adult recordings, reactance is usually several times larger than resistance. The admittance phase angle[h] at high negative pressure was found to be low at all frequencies. This suggests that the volume of air in the ear canal does not behave as a pure stiffness element. However, positive tail phase angles were above 70°, this would suggest a predominantly stiffness element at these pressures.

It is well established that, in adults, the number of extrema on the susceptance and conductance tympanograms increases with increasing frequency. Neonate tympanograms have more complex patterns than adults at similar frequencies. Table 3.1 shows the classification under the Vanhuyse[10] system for tympanograms recorded at four frequencies (as reported by Holte *et al.*[13]). An additional pattern was found at higher frequencies. The 0B1G pattern displays a monotonic rise in susceptance from negative to positive pressure with no identifiable extrema. An 'Other' category was also included for the large variety of shapes found (at higher frequencies) that did not fit any other category.

At 226Hz, 98% of tympanograms (+/-)[i] in the first week after birth were described by the Vanhuyse system (1B1G, 3B1G, 3B3G or 5B3G). With increasing frequency, patterns became more complex. At 450Hz, the most common patterns were 5B3G and 'Other'. At 710 and 900Hz, the majority of tympanograms obtained in the first three weeks after birth could not be defined by the Vanhuyse system (at 900Hz, no tympanogram in the first week after birth could be classified). Exceptions to this frequency relationship were identified. At 355 and 450Hz, patterns tended to be more complex than surrounding frequencies (226 and 710Hz). It was suggested that this was consistent with the presence of a resonant frequency at 450Hz. Holte *et al.*[13] investigated the developmental changes in the first 4 months after birth. They found that patterns became less complex. They concluded that preterm infant patterns would therefore be similar, or more probably, of increased complexity than patterns found for term newborns.

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[h] The phase angle relates the relative values of susceptance and conductance. Refer to Appendix B for explanation of this relationship.

[i] Refers to the direction of the pressure sweep. In this case it is from positive to negative pressure.

3.6.3 Developmental Changes in Tympanometry

Tympanometric assessment with children and neonates has become more popular with the advances in multi-component, multi-frequency immittance measurement instruments. Neonatal tympanograms often display double peaks, even with low probe tone frequencies (226Hz), indicating that the neonatal ME system is more influenced by mass and resistance than the adult ME system. It has also been suggested that hypomobility of the canal wall in infants confounds tympanometric results. Holte *et al.*[13] studied the maturation of tympanograms in neonates in comparison with infants and adults. They observed susceptance, conductance, admittance, as well as ear canal mobility. Twenty-three healthy full-term newborns were examined with conventional commercial equipment. They also utilized video otoscopy to observe ear canal movement. They concluded that canal wall movement was not the sole factor for atypical tympanogram shapes in neonates. The following points are age-related changes in neonate tympanograms as proposed by Holte *et al.*[13] :-

(a) mass and resistive components of the TM and ME system are more pronounced for neonates.

(b) related to the finding in (a), phase angle increases with age reflect a greater role of the ME stiffness component.

(c) resonant frequency of the ME system increases as a function of age.

(d) admittance magnitude increases with age.

(e) direction of air pressure change is an important factor.

(f) with infants <1 month old, a positive-to-negative air pressure change is preferable. The negative-to-positive direction often causes ear canal collapse.

(g) admittance values were found to have a high level of intersubject variability in the neonates tested. They questioned the usefulness of normative data with probe tones above 450Hz.

(h) at <4 months old, they recommend multi-component tympanograms only at 226Hz. Higher frequencies produce highly variable results which are difficult to interpret.

3.7 Data Analysis

3.7.1 Compensated Static Immittance

Static acoustic immittance data refers to values at specific ear canal pressure. There are two conventional ear canal pressures used for static immittance analysis; ambient ear canal pressure and tympanometric peak pressure (point of minimum impedance, maximum admittance). Ambient ear canal pressure would seem to be the obvious value to use considering that we use our ears at ambient pressure. However, there are practical problems associated with using ambient ear canal pressure; test-retest reliability[14] and intersubject variability[15] are excessively high. Tympanometric peak pressure is found to be constantly changing due to dynamic gas exchanges occurring in the ME. Ambient ear canal pressure does not, thus, identify the minimum impedance pressure of the ME. Shanks and Lilly[1] found that admittance magnitude (at 220 and 660Hz) was 24% lower at ambient than at peak pressure, the mean ear canal pressure between these two points varying by only 8daPa. This large difference over such a small pressure variation is due to the steepness of the tympanogram slope in this region. Wilson *et al.*[16] reported that static admittance at 0daPa could be as much as 33% lower than at peak.

Tympanometric peak pressure is often misinterpreted as a precise estimate of the pressure in the ME. However, peak pressure is a reasonable estimation of the pressure required that results in equal pressure on either side of the eardrum. It can, however, overestimate the ME pressure by as much as 100%[17]. It is, therefore, not possible to measure exactly the pressure in the ME with tympanometry. However, the location of peak pressure does provide important information on ME status.

Compensated static immittance is measured by subtracting the estimated ear canal volume from the peak immittance value gained during tympanometry. Errors can occur if only the vector magnitude information is used (single-component instruments) without observing the phase angle. Static immittance is influence by age and gender, it increases with age from 1 to 35 years, this increase being greater for males than females[16]. However, Van Camp *et al.*[6], pointed out that there is little unified normative data on static immittance. It has been shown that static immittance is useful in the identification and differentiation of most ME disorders. Fixation, for example, of the

ossicular chain reduces the mobility of the ME system. This causes the static susceptance to be reduced. However, there is a region for normal and fixed ears where static susceptance values overlap. This means that static immittance assessment on its own can be clinically inconclusive. Combined with other audiometric tests, it can be a useful clinical tool. For research or where data requires plotting over time it is essential to produce numerical forms of data. The methods of data presentation will be examined in the results chapter.

3.7.2 Sweep-frequency Probe Tones

An alternative to plotting tympanometric data with varying pressure is to measure immittance as a function of probe frequency (with constant pressure). Funasaka and Kumakawa[18] tested with a sweep probe tone frequency (220-2000Hz), during the sweep sound pressure and phase angle measurements were made. Sweeps were carried out at -200 and 0daPa. They plotted the results as frequency-sound pressure and frequency-phase angle curves. This system was established for the better detection of ME abnormalities. Location of the resonant frequency using this method is useful in assessing ME function.

This process is incorporated into the GSI33 instrument, a frequency sweep is performed (250-2000Hz) at 50Hz intervals. An initial sweep is conducted at +200daPa, a 226Hz admittance tympanogram is then produced to establish peak pressure location. A second sweep is then obtained at this peak pressure, comparison of admittance (default is susceptance) and phase angles at these two pressures is plotted against frequency. However, from initial testing for this current study, each sweep takes approximately 10 seconds to perform. The multiple sweeps cannot be interrupted. This was found to be impractical with the neonates initially tested. This plot will, therefore, be constructed from the complete range of tympanograms at a later stage.

3.7.3 Tympanogram Gradient

Tympanogram gradients can be used to supplement measures of ear canal volume, pressure for peak admittance and static admittance. The Gradient pressure difference (G_{dP}) index is easy to calculate and can contribute information not available from simple static admittance measures. It has been suggested that the G_{dP} may be useful in the detection of high impedance (low admittance) ME disease. Tompkins and Hall[19] investigated the use of tympanogram gradients in 80 normal and 80 otologically diagnosed otitis media subjects. They calculated gradients using the "ASHA Guidelines for Screening for Middle Ear Disease and Hearing Loss" method and by the system incorporated in the GSI33 Middle Ear Analyzer. The GSI33 instrument mean gradient value for normal ears was found to be 0.53, with otitis media ears being much lower at 0.20. However, it was noted that the GSI33 did not produce gradient calculations where a tympanogram peak was not identified. This situation is present in approximately one-third of otitis media ears, this is obviously an important limitation. Calculations for an additional 20 tympanograms were impossible using the ASHA method.

De Jonge[20] examined 80 students with normal ME function using three gradient methods; G_{dif}, G_{ratio} and G_{dP}[j]. He found that G_{dif} was a poor choice for gradient having little usefulness in discriminating between ears with and without effusion. Values correlated highly with static admittance, thus producing little additional information. Either of the other two methods, G_{ratio} and G_{dP}, was found to be acceptable. This method of quantifying the characteristics of tympanogram morphology will be considered during initial testing. Although, it is questioned whether this analysis is appropriate for the data that will be collected during this study.

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[j] The mathematical aspects of G_{dif}, G_{ratio} and G_{dP} will not be covered until an appropriate method has been chosen.

3.7.4 Plotting Strategies

As well as the basic plotting methods of susceptance and conductance (admittance) tympanograms, the corresponding reactance and resistance (impedance) tympanograms can be produced using the conversion equations (11) to (14) in Appendix B. Overall admittance (and impedance) can be calculated using the Pythagorean theorem to give magnitude and phase angle data. This alternative to rectangular notation (susceptance and conductance) is referred to as polar notation (admittance magnitude and phase angle).

The admittance magnitude and phase angle can be plotted for varying pressure, as admittance and phase angle tympanograms. Van Camp *et al.*[21] reported on the usefulness of the phase-angle tympanogram. They noted that notching occurred in the 678Hz phase-angle tympanogram for a mass related ME pathology. It has been suggested that phase-angle tympanograms may be superior to the individual susceptance and conductance tympanograms because phase is a ratio of both admittance components. Alternatively, instead of plotting susceptance and conductance (against pressure) tympanograms separately, these can be combined (susceptance versus conductance plot). This phasor diagram was originally introduced by Creten *et al.*[22]. They highlighted that the height-width relationship of the phasor plot was of particular importance for the diagnosis of ME pathologies.

With the use of computer interfacing, a multitude of plotting formats can be considered. The GSI33, in use for this study, is linked by an RS232 serial interface with an IBM compatible PC. Test details and data are transferred after each tympanometric run, the full numerical data can then be processed and analyzed at a later stage.

3.8 Protocol Selection

Test variables are vitally important for the application of tympanometry. The areas to be considered are; the pressure direction (positive-to-negative versus negative-to-positive), rate of pressure change (eg. 25, 50, 100daPa/second) and the number of trials for a subject while the ear canal remains scaled[23]. A suitable pressure

range must be established to prevent disturbance of the subject. The most appropriate test frequencies will also be identified.

3.8.1 Direction of Air Pressure Change in the External Ear Canal

The appearance of the tympanogram can be altered with pressure change direction, an ascending (negative-to-positive) direction causing a higher incidence of notching. Peak static immittance readings, phase angles and peak pressure values are also affected. Hall and Chandler[24] analyzed 182 patients undergoing routine tympanometric assessment, admittance tympanograms were recorded using a Grason-Stadler Middle Ear Analyzer. A probe tone of 226Hz was used with a pressure change rate of 50daPa/second. Separate tympanograms were recorded for positive-to-negative (descending) and negative-to-positive (ascending) pressure directions. Descending pressure started at +200daPa with ascending starting at -300 or -400daPa. They found that pressure direction could influence both peak position and amplitude. The average peak difference was reported to be 31.2daPa between the two directions. Ninety-seven percent of ears showed a more negative peak with a descending direction. Amplitude changes with direction were found to be minimal, the majority of ears (62%) having a lower amplitude for descending than the ascending pressure change (21% lower for ascending than descending).

3.8.2 Rate of Ear Canal Pressure Change

The primary pressure rate effect is on static immittance, peak static admittance decreasing as the rate of pressure change is increased[25]. The non-linear behaviour of the ME is thought to increase admittance values, whereas the temporal response of the recording instrument at higher rates can produce measurement artifacts resembling rate effects. This has the effect of reducing recorded admittance values. The effect of pressure rate on peak pressure location is not conclusive, some claim no effect[26], whilst others suggest that peak pressure location is a function of rate[25].

Obviously, there is an advantage of using a faster rate, individual tests being quicker to perform. This is particularly desirable when testing neonates. Holte *et al.*[13]

tested full-term newborns, they used a rate of 250daPa/s and found this highly beneficial in this particular population. The equipment for this study can vary pressure at rates of 12.5, 50, 200 and 200/600[k]daPa/s. From initial testing, it was obvious that a faster rate is beneficial for testing the neonate population. For this reason, a pressure change of 200daPa/s is stipulated. This is deemed more reliable than the 200/600daPa/s combination run.

3.8.3 Pressure Range

The pressure range specification has two automatic settings, normal (-400 to +200daPa) and broad (-600 to +400daPa). The relatively large negative pressure selection of the broad range specification is unsuitable for neonate ears. Preliminary tests were performed with the normal range specification to establish whether the negative value (-400daPa) was acceptable, this value was found to be well tolerated. The starting positive pressure of +200daPa was found to be suitable.

3.8.4 Sweep Frequency

The commonly used recording frequencies for adult testing are 226 (220), 678 (660) and 1000Hz. The GSI33 instrument allows for recording in diagnostic mode at 226, 678 and 1000Hz. These tests will be performed first. Where the infant is non-cooperative, it is hoped that these tests can still be recorded. The multi-frequency option on the GSI33 allows recording at frequencies between 250 and 2000Hz at 50 Hz intervals. Initial runs with this option attempt to identify the resonant frequency of the ME system. The probe tone is automatically swept from 250 to 2000Hz in steps of 50Hz at a constant pressure (+200daPa). Susceptance (B), conductance (G) or Admittance (Y) values and phase angle measurements are recorded for each frequency. A 226Hz tympanogram is then obtained to locate the peak admittance pressure. The frequency sweep is then repeated at this pressure. Data is then displayed as the difference in susceptance (or conductance, admittance) and phase angle between peak and +200daPa,

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[k] The 200/600 option allows for faster testing (600daPa/s) during the negative region of the pressure sweep, the positive region being tested at 200daPa/s.

this data is plotted against probe tone frequency. Resonant frequencies are identified at points where the change in susceptance (ΔB) between the two sweeps is equal to zero. The user can then record tympanograms (B, G or Y) at any frequency between 250 and 2000Hz (50Hz steps). The rate of frequency sweep for these initial tests is not defined by the user, and takes approximately 10 seconds for each run. Initial tests have shown that this will be an impractical length of time to keep a hermetic seal with the majority of infants. It is, therefore, proposed to bypass this initial procedure. A less detailed susceptance (and phase angle) versus probe frequency plot can be calculated post-test from the tympanograms generated at various frequencies.

3.8.5 Selection of Probe Tone Frequencies

Most recordings will be concentrated in the 226 to 1000Hz frequency range, this range being more commonly used and more mathematically stable. Tympanograms will also be obtained in the 1000 to 2000Hz range, these tend to be more complex and difficult to interpret. Holte *et al.*[13] recorded neonate tympanograms at 226, 450, 710 and 900Hz in a random order. With exceptionally cooperative subjects, 355 and 560Hz were also recorded. Shanks *et al.*[27] collected tympanograms at 226, 339, 452, 565, 678, 791, 904, 1017, 1130, 1243Hz (half multiples of 226).

With the equipment in use for this study, it is proposed that both susceptance (B) and conductance (G) tympanograms will be recorded at 226, 300, 400, 500, 600, 678, 800, 900, 1000, 1250, 1500, 1750 and 2000Hz. Tympanograms are collected at 226 and 678Hz instead of 200 and 700Hz because of their standard use. This will aid in the comparison of data with other studies.

3.9 Conclusion

The mechanical properties and mathematical concepts concerned with the production of tympanometric data are presented in Appendix B. The effects of the ear canal were identified and the necessary procedures for the correction of data to the plane of the tympanic membrane introduced. Test procedures have been investigated to ensure reliable and repeatable collection of tympanograms. Currently, test protocols found in the clinical environment are limited to single-frequency, single-component testing. This study will measure the two components of admittance (susceptance and conductance) at various frequencies from 226 to 2000Hz. Other parameters (admittance magnitude, phase angle and impedance components) can be calculated from the measured data utilizing the mathematical concepts described in Appendix B.

The clinical use and characteristics of tympanometric data from the neonate population was assessed. There are currently limited reviews of data during the term, and especially the preterm periods. Various descriptive procedures have been investigated, several schemes would appear to be of use. Preliminary testing, in conjunction with this review, has shown considerable variability in tympanogram morphology. This will obviously affect the selection of particular descriptors. The data obtained would suggest that tympanogram gradient, phase angle tympanograms and phasor plotting strategies are not suitable for analysis.

A detailed test protocol has been established, this being constrained by the particular population and the time limits of the test sessions. The direction of air pressure change in the ear canal is dictated by the population, a positive-to-negative direction being essential to avoid ear canal collapse in the infant. A compromise has been identified for the rate of the pressure change. A low rate is optimal for tympanogram definition. However, the preliminary testing showed that the time for which an hermetic seal could be sustained is short, particularly with restless subjects.

The use of the automated procedure for middle ear resonance identification (on the GSI33) was rejected. Sustaining an hermetic seal for the sweep-frequency routine (10 second per run), which requires multiple runs without any leakage, was found to be impractical with this population. This facility is not optional on the GSI33 and will, therefore, be run in an enclosed chamber. Probe tone frequencies were selected in

addition to the commonly used 226, 678 and 1000Hz. The investigation will concentrate on the 226 to 1000Hz range, data generally being less complex and more mathematically stable than the 1000 to 2000Hz region (some testing will also be carried out in this higher region).

The review of previous tympanometric studies involving preterm infants suggests that tympanogram acquisition is possible from this population. However, a relatively high degree of variability is expected. A number of procedural problems were identified during initial testing. These were predominantly concerned with restless incubated infants. The reliability and repeatability of tympanometric data from the infant population has been questioned. However, knowledge of the expected tympanogram patterns will assist identification of less well defined data.

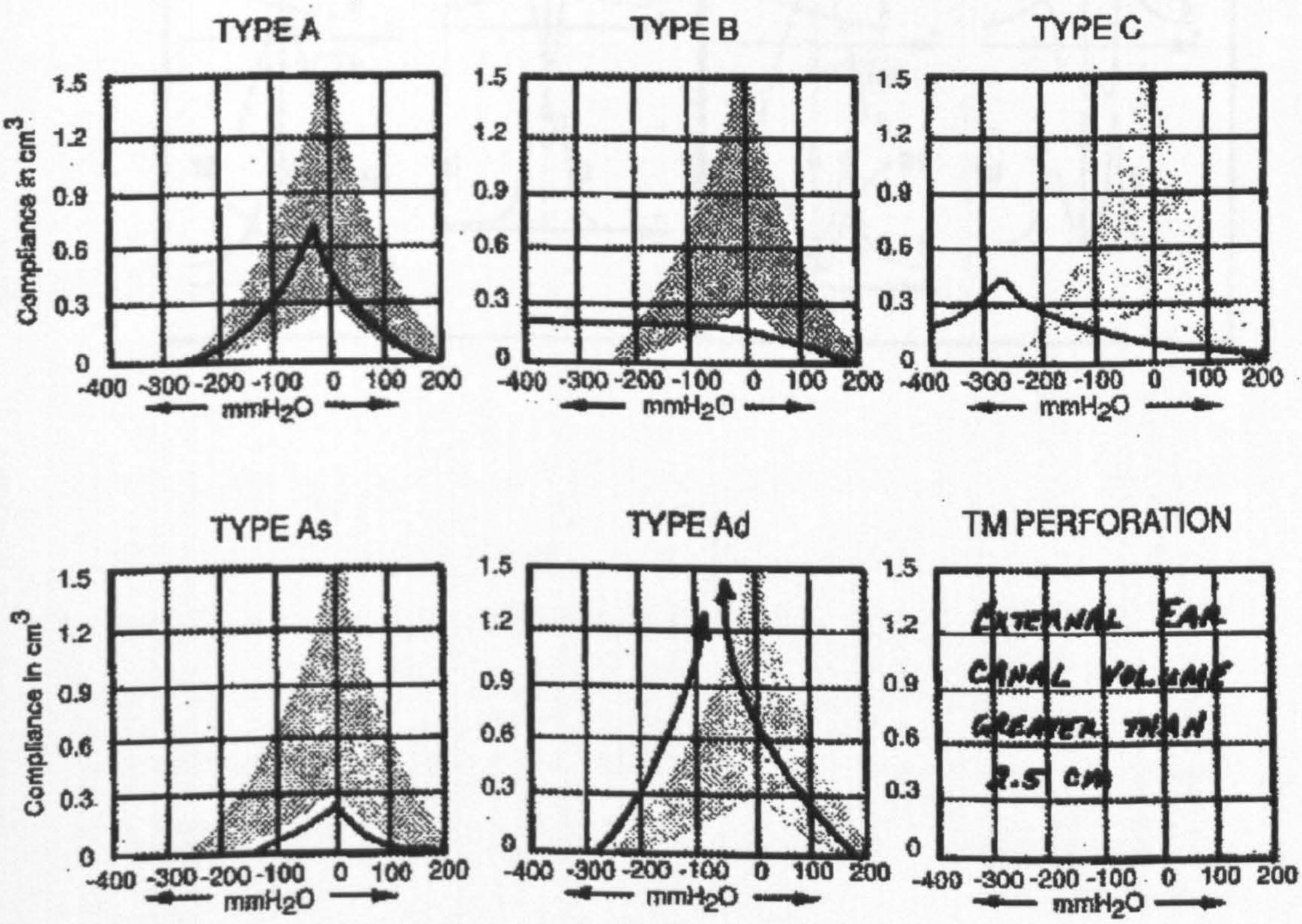
3.9.1 The Acoustic Reflex

Testing of the acoustic reflex was performed in conjunction with the tympanogram acquisition. This testing was limited due to time limitations and procedural difficulties. The collection of contralateral reflex data, which is of interest when assessing neurological function, was not possible. A limited amount of ipsilateral reflex data was collected. These results are presented in Appendix E for reference. They will not be discussed in this thesis.

Table 3.1. Percentage of tympanometric types classified under the Vanhuyse model (data from Holte *et al.*[13])

Age: 1-7 days	*226Hz*	*450Hz*	*710Hz*	*900Hz*
1B1G	28	4	4	0
3B1G	19	19	4	0
3B3G	23	19	0	0
5B3G	28	38	0	0
0B1G	0	0	0	0
Other	1	19	92	100

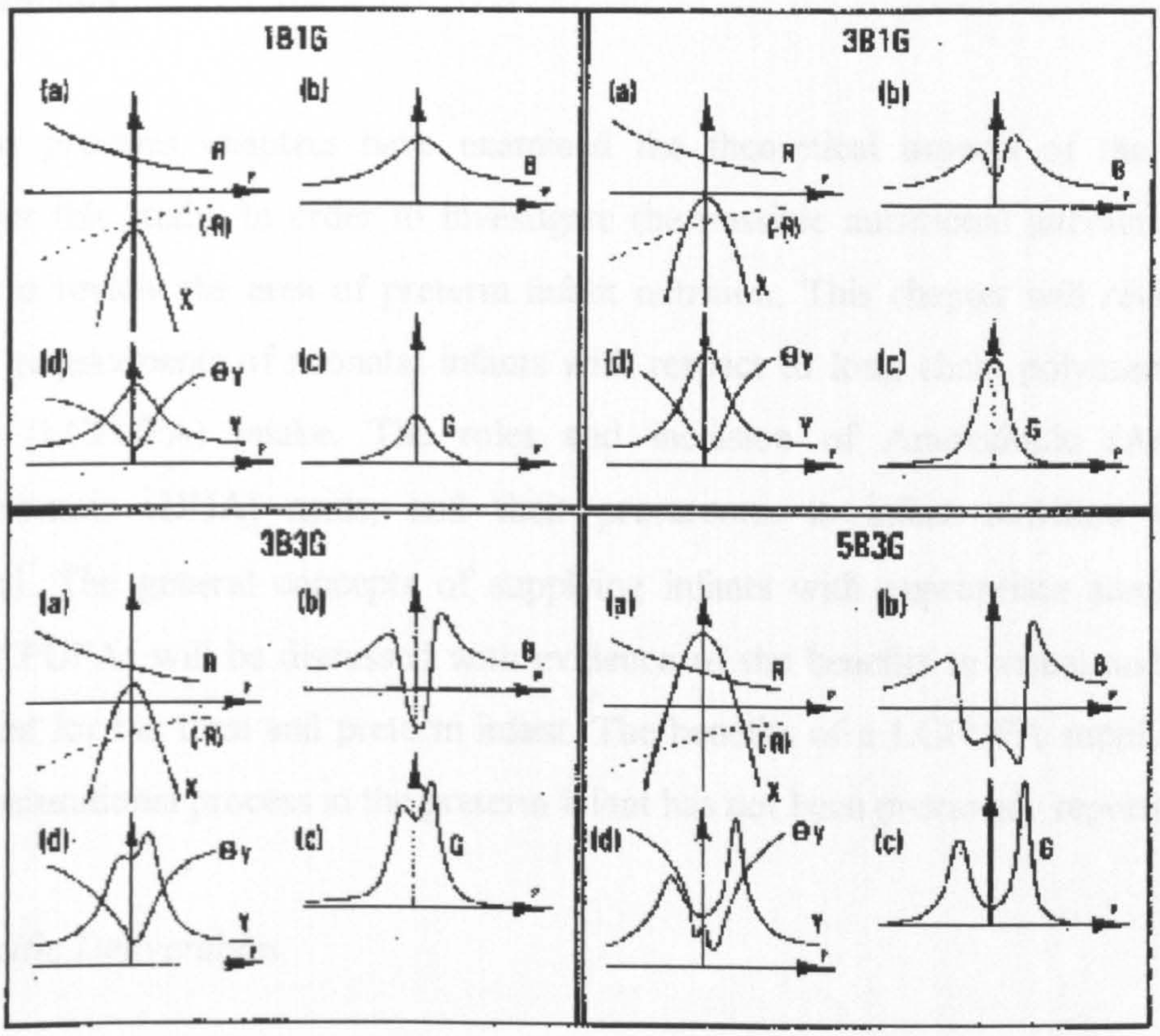
Figure 3.1 Jerger[4] classification system for commonly encountered tympanogram shapes for admittance (Y) at low frequency (eg. 220Hz)



(Pressure values in mmH_2O are approximately equal to daPa units)

Shaded region shows range of normal characteristics

Figure 3.2 Vanhuyse[10] model for description of tympanograms using the number of extrema present in susceptance (B) and conductance (G) patterns The susceptance and conductance tympanograms are shown in plots (b) and (c). Plot (a) shows the corresponding impedance tympanograms, and plot (d) the admittance (and phase angle) tympanograms.



CHAPTER FOUR

INFANT NUTRITION

4.1 Introduction

The previous chapters have examined the theoretical aspects of the testing available for this study. In order to investigate the possible nutritional interaction it is necessary to review the area of preterm infant nutrition. This chapter will review the nutritional requirements of neonatal infants with respect to long chain polyunsaturated fatty acid (LCPUFA) intake. The roles and inclusion of Arachidonic (AA) and Docosahexaenoic (DHA) acids, and their precursors, in infant nutrition will be investigated. The general concepts of supplying infants with appropriate amounts of specific LCPUFAs will be discussed with evidence of the benefits to visual and neural development for the term and preterm infant. The benefits of a LCPUFA supply to the auditory maturational process in the preterm infant has not been previously reported.

4.1.1 Specific Deliverables

- To identify the mechanisms of nutrient uptake in the neonatal period.
- To investigate the role of the AA and DHA precursor acids.
- Familiarization with the terminology of infant nutrition.
- To establish the role of formula milks including a supply of LCPUFAs.
- To identify the potential role of LCPUFAs on the preterm infant.
- To review previous studies on the effect of LCPUFAs on visual and neural development in the neonate.

4.2 Foetal Uptake of Nutrients

Fatty acids are transferred across the placenta according to maternal plasma levels. These acids are used for structure and energy storage rather than for an immediate energy supply. Foetal free fatty acid composition differs from maternal in one way, the foetal fatty acid has a larger quantity of AA than the maternal supply. It is thought that this level is produced by linoleic acid (LA) in the placenta; the maternal lipid stores and diet contain very little. Umbilical uptake of nutrients depends on placental function and umbilical blood flow. The foetus and placenta must be considered together with respect to nutrition. Foetal tissue growth is entirely dependent on the placental exchange of nutrients and gases. AA and DHA are derived from the essential fatty acids (EFA) linoleic (LA) and α-linolenic (ALA) acids. Their production is vital, AA and DHA have an important structural role as components of cellular and subcellular membranes, particularly in nervous tissue.

Modern commercially available artificial formulas tend to leave the preterm infant with a poorer LCPUFA status than infants fed mothers' milk[1]. This has been attributed to the small but significant amounts of DHA, AA and other long chain polyunsaturates (LCP) in human milk. Formula milks contain substantial levels of the parent EFAs, LA and ALA. These can be converted to AA and DHA by the body. It is known that the bodily processes of the preterm infant cannot guarantee an appropriate supply of LCPUFAs solely through metabolic conversion of the parent EFAs[2].

The three fatty acids required by the body have to be obtained from the diet; LA, AA, and ALA. LA is the most important because it is the major EFA component of lecithin, the principle phospholipid in the membrane structure of all cells. AA can be synthesized by the body from LA. Each of these PUFAs is readily oxidized in air and easily destroyed in food unless stabilized by an antioxidant, such as vitamin E.

4.2.1 The Role of AA and DHA in Infant Nutrition

AA and DHA are important structural components of cell membrane phospholipids[a]. AA is widespread throughout, the body with high concentrations in the brain and vascular endothelium[b], it is quantitatively the most important LCPUFA. DHA, however, has a highly specific distribution being the predominant membrane fatty acid of synaptosomes[c], retinal photoreceptors, and mitochondria[d], but being scarce in other tissues. During foetal life LCPUFAs are actively transferred across the placenta to the foetus. The concentration of circulating LCPUFAs in plasma phospholipids is higher in newborns than in their mothers.

Human breast milk is considered to provide all the fatty acid needs for optimum growth and development of a healthy term infant. With infants born before 33 weeks gestational age (GA), the immaturity of the Central Nervous System (CNS) alters the biochemical and functional processes that are susceptible to nutritional insult[3]. Innis[3] suggested that a dietary source of DHA can increase the rate of maturation of retinal function and visual acuity. However, there remains no conclusive evidence that a dietary supply of AA or DHA will improve neurodevelopmental outcome in very premature infant.

4.3 Basic Biochemistry and Terminology

Lipids are transported in serum as macromolecular complexes with protein. Fatty acids, which constitute the energy source in lipids, differ in respect to chain length (8 - 10 carbon atoms being medium-chain, 12 or more being long-chain) and the presence or absence of double bonds (2 or more such as LA being termed polyunsaturated). The majority of fatty acids are esterified with cholesterol (cholesterol esters), with glycerol (triglycerides), or with glycerol, phosphorus and bases (phospholipids).

Lipid, acting as a vehicle for fat, is not only useful in supplying a concentrated source of calories, but also prevents EFA deficiency. Lipid emulsions for intravenous

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[a] Phospholipids - a lipid containing a phosphate group and one or more fatty acids.
[b] Endothelium - a layer of cells forming a lining.
[c] Synaptosomes - a synapse (a junction of two nerve cells) pertaining to the sensory structures of the body.
[d] Mitochondria - a filamentous structure commonly called the "powerhouse of the cell".

feeding commonly have >50% of the fatty acids as LA. EFA deficiency is unlikely to occur unless lipid is completely excluded. Concerns that this high concentration of LA would reduce synthesis of AA have not been supported by recent studies by Cooke *et al.*[4]. It is still not clear whether the same applies to the long chain derivatives of ALA which are essential components of structural lipids for the nervous system and blood vessels. The brain is 60% lipid and has an extremely high energy consumption. The foetus does not use this fat supply, but stores it so that the fat can provide the EFAs required for postnatal neural growth. These acids are termed fatty acids, and include AA and DHA. The last trimester of pregnancy, in the term infant, is when the main neural cell expansion and development takes place[5]. Obviously this period is very important for the preterm infant in the extra-uterine environment.

Approximately 50% of the total fatty acids in the brain's grey matter consists of AA and DHA[6]. Clandinin *et al.*[7] showed that 80% of intra-uterine AA and DHA are accumulated during the last trimester. Preterm infants in the extra-uterine environment are currently not supplied with these levels of AA and DHA. Brain growth before birth is concerned with the maternal diet. Around 70% of brain cells to last an individual's life span have divided before birth, the most active period being within the first few weeks of embryonic life. This development is highly dependent on the mothers' health as the placenta is not yet formed. When the placenta is formed, the foetal brain will consume 70% of the dietary energy, 60% being extracted from the mothers' milk after birth.

4.4 Detecting Levels of Fatty Acids

The LA and ALA content of human milk is dependent on the maternal intake. Clandinin *et al.*[8] found preterm human milk to contain 11.3% and 0.3% of total fatty acids as LA and ALA respectively. Van Beusekom *et al.*[9] studied the fatty acid composition of plasma cholesterol esters (CE), erythrocytes (red blood cells (RBC)) and mature milk from mothers of breastfed newborns. At birth, cord blood plasma CE and RBC LCPUFA content was found to be higher, and LA and ALA acid content lower, than corresponding maternal compartments. DHA content was lower and AA content higher in cord blood RBC than in maternal RBC. With breast feeding, LCPUFA plasma CE content dropped with RBC LCPUFA content remaining virtually constant. They

concluded that RBC LCPUFA content is a more reliable parameter for assessing LCPUFA status.

4.5 Formula Milks containing LCPUFAs

It has for a long time been known that LA is essential for the growth and development of infants. It is now common for formula feeds to contain this AA in levels similar to those found in breast milk. Until recently levels of DHA precursor, ALA, has had little attention. Most infant formulas contain <1% ALA, with LA:ALA ratio's ranging from 8:1 to more than 20:1[10]. Carlson *et al.*[11] observed that the erythrocyte membranes of preterm infants fed formula with high LA:ALA ratio (>19:1) are depleted in DHA relative to those fed breast milk. Due to the numerous production problems with including a source of DHA in formula milks, research has been carried out into maintaining DHA levels in erythrocyte membranes by providing an adequate supply of ALA. Gibson *et al.*[12] used two formulas (LA:ALA ratio 4:1), one with increased ALA, one with a reduced amount of ALA. These were compared with a standard formula (high LA:ALA ratio 19:1) and breast milk. Results showed that ALA levels in formula feeds were well reflected in erythrocyte membrane levels. The erythrocyte membrane levels of some acids were greater than those found with breast feeding. However, none of the formulas increased the levels of DHA above that of breast feeding. Gibson *et al.*[12] point out that the use of DHA from marine sources in infant formulas has not been fully evaluated in safety terms, and that further research is necessary into other ways of supplementing appropriate DHA levels or for increasing conversion of ALA to DHA.

Chirouze *et al.*[13] studied the fatty acid composition of RBC phospholipids in low birthweight infants (LBWI). Levels were recorded for the first 3 months of life. In one of their studies, infants were fed one of two formulas, one supplemented with LCPUFAs and one unsupplemented. They concluded that adding DHA to formulas was more effective than increasing the ALA content in maintaining RBC phospholipid DHA levels. They found that infants supplemented with LCPUFAs kept their DHA level stable in RBC phospholipids. Unsupplemented infants were found to have decreased levels. Carlson *et al.*[14] confirmed that erythrocyte DHA is higher in breastfed compared to formula fed infants, both term and preterm. It is accepted that this is due to an absence of

DHA in formulas containing vegetable oil as a source of fat. ALA has not been as effective in increasing cellular DHA as feeding even small amounts of DHA. In a previous study, Carlson *et al.*[14] found that formula containing 2.5% energy from ALA and 0.1% energy from DHA resulted in twice the amount of erythrocyte phospholipid DHA as a feed without a direct DHA content.

4.6 Prematurity

As previously mentioned, the main structural material of the brain is lipid, these lipids have a unique profile of LCPUFA which are EFAs. An expert consultation[15] argued the case that LCPUFAs should be included in infant formulas, with human milk being used as a guide with regard to formula composition. Preterm infants are denied the benefits of the placental system that selects LCPUFAs for the developing foetus, the brain of which is developing rapidly. In general, clinical evidence shows that common disorders with preterm infants are closely associated with membranes in which AA and DHA are of critical importance[16].

Although the essentiality of LCPUFAs in infant nutrition is established, controversy over many basic questions relating LCPUFA to neonatal development still exist. The antenatal accretion of LCPUFA occurs mainly during the last trimester of gestation. Hence, a preterm infant will be born with little or no LCPUFA resources. It has been suggested[17] that LCPUFA supply to the foetus occurs to the extent that the maternal circulation of LCPUFA is deficient. This might suggest that dietary supplementation during pregnancy would be beneficial[17]. It has been found that certain nutrients, AA and DHA among them, could be important in preventing retinopathy and blindness, and also encourage complete cognitive development. Uauy *et al.*[18] demonstrated that a supply of DHA in formula for very LBWIs was necessary to sustain retinal rod function similar to breastfed infants.

The preterm infant is born at a time when all membrane development is unprepared for the extra-uterine environment. Current feeding regimes can further compound nutrient deficiency by not supplying the levels of EFAs normally provided by the placenta. Breast milk contains a range of LCPUFAs, formula feeds however are only fortified with precursor EFAs such as LA and ALA. Evidence does exist that formula-fed

infants are unable to metabolise their full requirement of LCPUFA from these precursors since they have less DHA and AA in their erythrocytes than breastfed infants[19]. Makrides *et al.*[20] recognised that deficiency of fatty acid in infant nutrition is especially important with preterm infants because of the spurt in brain growth during the last trimester.

4.7 Visual and Neural Development

Makrides *et al.*[20] observed neural maturation in 79 healthy term infants utilizing visual evoked potentials (VEP); electroretinograms[e] and psychometric tests. VEPs were carried out at 16 and 30 weeks after birth. They found that breastfed infants had better VEP acuities than reference formula-fed subjects. Infants fed with supplemented formula had acuities matching those of the breastfed group. They also noted that at enrolment (day 4-6 after birth) breastfed infants already had higher levels of DHA and AA, and lower levels of LA than those fed formula. They found that only erythrocyte DHA correlated with VEP acuity at 16 and 30 weeks, no other fatty acid consistently correlated with VEP acuity. In conclusion, Makrides *et al.*[20] recommended a 0.36% DHA supplement that would fully match VEP acuity of purely breastfed infants.

Farquarson *et al.*[21] reported that brain DHA is higher in human milk fed rather than formula-fed infants. Carlson *et al.*[22] reported direct evidence that dietary DHA was conditionally essential for visual development in preterm infants. Carlson *et al.*[22] have completed a series of studies over the last 10 years on the effects of the DHA dietary source on LCPUFA levels, visual acuity, growth and development. They experimented with a formula containing 0.2% DHA with 0.03% eicosapentaenoic acid (EPA). They fed preterms until 2 months past expected term date. They found that a marine oil source of DHA containing EPA was not optimal for improving DHA status. Also that AA status was found to be poor in the formula fed infants. It was concluded that the marine oil directly reduced growth achievement and AA status, resulting in poorer psychomotor[f] development. However, this was overcome by reducing the EPA content and only feeding DHA until 2 months past term. Results showed that formula infants had better visual acuity at term and at 2 months, although from 4-12 months results were similar to

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[e] Electroretinogram - an electrophysiological test for retina function.
[f] Psychomotor - concerning the study of movement resulting from mental activity.

breastfed infants. It was found that DHA content in RBC phospholipids began to decline after the 2 month study period to normal levels. However, the formula fed infants achieved higher Bayley Mental Developmental Index scores at 12 months. Carlson *et al.*[22] concluded that this was evidence of the need for early exposure to DHA for early mental development.

Birch *et al.*[23] studied the effect of DHA supply on visual acuity development in very LBWIs (born 27-33 weeks PCA) using VEPs at 36 and 57 weeks PCA. Infants were given one of three diets; corn oil providing solely LA, soy oil providing LA and ALA, or soy/marine oil providing the same as soy oil but with the addition of preformed DHA. Soy/marine oil infants were found to have higher DHA levels in erythrocyte membranes and better VEP acuity at 37 and 57 weeks PCA than the corn oil fed group. The soy oil (only) infants had intermediate DHA levels with significantly poorer VEP acuity at 57 weeks PCA than the soy/marine oil group. Only the soy/marine oil group had VEP acuity comparable to that of breastfed infants.

Koletzko *et al.*[24] studied formula supplementation with 27 LBWIs. Both LA and ALA were included in formula milk at levels similar to those found in human milk. Infants were either breastfed (with fortified proteins and minerals), or randomly assigned to formula milk (Prematil, Milupa AG, Friedrichsdorf, Germany), one supplemented and one not supplemented with LCPUFAs. They found that infants fed formula without LCPs had a significant depletion of plasma phospholipid AA (74% of breastfed) and reduction of DHA (64% of breastfed) within the first 3 weeks of full enteral feeding. However, infants receiving LCP enriched formula achieved LCP status equal to that of the breastfed infants. They concluded that LBWIs can absorb formula LCPs and incorporate them into endogenous phospholipids. There were no adverse effects of LCP enrichment on vitamin-E status, the milk was well tolerated, supplied sufficient amounts of AA and DHA, and did not interfere with short term growth.

Agostoni *et al.*[25] observed neurodevelopmental performance in term infants, Brunet-Lézine psychomotor development tests were carried out at 4 months. It was found that infants supplemented with LCPUFA had significantly higher scores than breastfed and unsupplemented infants in turn. AA and DHA levels in circulating lipids and erythrocyte phospholipids were found to be higher among the supplemented and breastfed groups.

Faldella *et al.*[26] investigated the influence of LCPUFAs, especially DHA, on healthy preterm infants examining VEPs, flash electroretinography (ERG) and the ABR[g]. The ABR testing was only performed at 52 weeks PCA. There was no discussion of maturational characteristics. Infants were fed from birth with breast milk and two formula feeds, one supplemented with LCPUFAs. Readings were then taken at 52 weeks PCA and correlated with fatty acid composition of RBC membranes. They noted that the VEP of the breast milk and LCPUFA supplemented infants showed similar morphology and latency characteristics. The non-supplemented group displayed a different morphology with longer latency values. This would suggest that the higher levels of erythrocyte LCP, especially DHA, led to a faster visual maturation by 52 weeks PCA. They found that ERG and ABR recordings were similar in all groups at the 52 week PCA stage of development. Faldella *et al.*[26] concluded that LCP supplement in preterm formulas can positively influence the maturation of VEPs in preterm infants when breast milk is not available.

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[g] The study concentrated on visual development.

4.8 Conclusion

It has been established that foetal nutrition is largely dependent on the mothers' ability to supply the necessary levels of LCPUFA, and other nutrients required for cell development. It is, thus, imperative to supply the preterm infant, in the extra-uterine environment, with LCPUFA levels similar to those normally produced by the mother. It has been found that these acids are important structural components of cell membrane phospholipids, the brain being 60% lipid. The preterm infant is particularly susceptible to neurological impairments, the last trimester of pregnancy being a period of accelerated cell expansion and development.

Previous research into neonatal blood chemistry has provided techniques for accurately and reliably assessing composition and levels of LCPUFAs. This has led to the identification of schemes for the introduction of these acids to the neonatal diet. Linoleic (LA) and α-linolenic (ALA) acids, the parent essential fatty acids (EFA) to both AA and DHA, are currently included in many formula milks allowing the neonate body to convert these to AA and DHA. However, the appropriate quantities of the precursors have not yet been fully examined.

Conclusions of previous research would suggest that preterm diet is an important factor when analyzing infant development. From this review it must be concluded that LCPUFAs, in the form of arachidonic (AA) and docosahexaenoic (DHA) acids, are beneficial to vital structures in both the term and preterm infant. The preterm period is a time of accelerated cell development. It is, therefore, essential to provide the preterm infant, deficient in LCPUFAs, with an additional supply of these nutrients. Faldella *et al.*[26] recorded the ABR at 52 weeks PCA in conjunction with LCPUFA supply, but found no difference between feeding regimes. However, this was a single test study that did not consider the maturational processes. Their data tended to concentrate on visual development.

This review has discussed the interaction between neonatal diet and neurological measures of visual acuity and the neurodevelopmental processes. Through this the need for LCPUFA supplementation has been established. Further research is required as to the optimal method of achieving appropriate LCPUFA status in the preterm neonate. It should be noted that the test formula for this current study is enriched with LCPUFAs, though these levels are not particularly high.

CHAPTER FIVE

AUDITORY BRAINSTEM RESPONSE (ABR) RESULTS

5.1 Introduction

In total 28 infants born between 37 and 42 weeks GA were recruited onto the term study. These infants were tested for ABRs within 23 to 130 hours after birth. Infants born through caesarean section were targeted due to them staying in hospital with the mothers until approximately 4 to 5 days after birth. Most were tested at 3-4 days of age to avoid the middle ear (ME) problems associated with the first day or so after birth[1]. The infants were equally divided for gender (14 male, 14 female). The test ear was chosen at random so that 7 tests were performed on each ear for each gender. The same recruitment procedure was used throughout, with subjects being approached at random regardless of age (within the term period), gender or birthweight.

The mean GAs for males and females were 38^{+4} * (n=14 S.D. 1^{+4}) and 39^{+2} weeks (n=14 S.D. 1^{+1}) respectively (group mean 38^{+6} weeks (S.D. 1^{+4})). The mean birthweights were 3148 (n=14 S.D. 641) and 3335g (n=14 S.D. 556) for males and females respectively (group mean 3243g (S.D. 597)). Analyzing birthweight for GA showed that the majority of infants were within 90% of the normal mean[2]. The distributions for GA and birthweight can be seen in Figures 5.1 and 5.2. The Pearson Correlation coefficient shows a significant correlation ($P<0.005$) between GA and birthweight. The Wilcoxon test was performed for gender differences for GA and birthweight, no significant differences were found ($P>0.05$).

A total of 26 infants fitting the criteria of <32 weeks gestational age (GA) or <1500g birthweight were recruited onto the preterm study. Three of these infants were later transferred to other hospitals or developed medical conditions which made them unsuitable for continuation on the study. A further infant was excluded due to poor (virtually non-existent) ABR results. It is not known why the elicitation of ABRs from this infant was not possible. There are many reasons why ABR elicitation is not possible

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* Refers to 38 weeks and 4 days of gestation.

when testing the preterm population. This case could be due to a transient ME condition or an actual hearing loss. It was also noted that some incubators produced high levels of interference, as did particular areas of the neonatal intensive care unit (NICU). This may be a factor in this case. This infant was referred for further continuing assessment at the Child Development Centre (CDC). Of the remaining 22 infants, twenty were tested for more than two sessions. The maximum number of test sessions performed on a single infant was five.

A plot of GA against birthweight can be seen in Appendix D. Eighteen of the infants were born <32 weeks, a further three being born <33 weeks PCA. Subject LOB, born 36^{+3} weeks, was recruited due to a birthweight <1500g. Mean GA and birthweight (excluding subject LOB) were 30^{+3} weeks (n=21 S.D. 1^{+4}) and 1327g (n=21 S.D. 329) respectively. The mean PCA for the first and final test sessions were 32^{+1} (n=21 S.D. 1^{+4}) and 37^{+6} weeks (n=21 S.D. 1^{+5}) respectively. The distributions for GA and birthweight for the preterm group can be seen in Figures 5.3 and 5.4.

The scatter plots, from which all L-A functions were constructed, can be found in Appendix C for term infants and Appendix D for the preterm subjects. These plots are presented for overall group behaviour and for gender, and in the case of the preterm group, dietary groupings. Summary L-A function plots are also presented within this chapter. These show functions for absolute latency, IPL, and the 37-13 and 61-13pps[b] differences (for absolute latency and IPL). These plots show the relationships between different parameters and stimulus rates. Numerical latency-age (L-A) function data for the term infants can be found in Table 5.1. Gender values are presented in Tables 5.2a/b. Overall means for the term period and predicted 40 weeks PCA values (from L-A functions) can be found in Tables 5.3 and 5.5 respectively. L-A functions for the preterm group are presented in Table 5.6, with gender data in Tables 5.7a and 5.7b. Tables 5.8, 5.9a and 5.9b show category mean data for two week periods from 28 to 40 weeks PCA. Table 5.10 shows broader category mean data for dietary groupings throughout the preterm period.

For reference, some example ABR data is reproduced in Figures 5.5 to 5.7. Figure 5.5 shows the interaction of intensity on the latency of waves I, III and V. Figure

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[b] 37-13 and 61-13 differences refer to the differences between the respective stimulus rates calculated from the raw data. Obviously, these trends may differ from those concluded from absolute latency trend data.

5.6 shows the rate effect for the stimulus rates used for this study. Figure 5.7 shows the maturation of data (13pps) for subject DEN (GA 30^{+2} weeks). Traces for five test sessions at 60dB nHL are presented.

Data for the ABR waves I, III and V, and the associated IPLs (ie. I-III, III-V and I-V) are presented. The majority of testing was performed at a base stimulus rate of 13pps. Data is presented for 60dB nHL for all ABR parameters. The L-I function data utilizes intensity levels of 80dB nHL down to threshold levels. The rate effect was studied using increased stimulus rates of 37 and 61pps. The results will be discussed and logical conclusions drawn ready for further discussion and comparison with previous research. The data presented uses latency-age (L-A) functions for groups of subjects. Other methods of data analysis considered and the choice of analysis is discussed in Chapter Seven.

As previously mentioned, full scatter plots are presented in Appendices C and D. The L-A functions constructed from group data are accompanied by 95% confidence bands. The influence of these bands and the relevance of the conclusions will be investigated in this and Chapter Seven. The one-sample t test was performed on all linear regression L-A functions. The significance of this test allows for investigation as to whether the slope of a L-A function is significantly different from a zero slope. This shows whether a maturational change observed in an ABR parameter is statistically significant. This test is equivalent to the significance test for the Pearson Correlation coefficient (r^2). These values are presented with data point numbers on the scatter plots in Appendices C and D.

Differences between slopes was assessed using the unpaired t test. This test was implemented for assessment of gender maturational differences and comparisons of different IPL maturational rates. Differences in preterm and term L-A functions were also assessed with this method. Comparisons of term data and preterm data acquired during the term period were examined using the unpaired Wilcoxon non-parametric test (this test is equivalent to the Mann-Whitney U test). The unpaired Wilcoxon test was also implemented to assess gender differences in both term and preterm data during the term period.

5.2 Base Stimulus Rate (13pps)

5.2.1 Term Data

5.2.1.1 Absolute Latencies

Latency values for wave I (13pps) range from 1.80 to 2.60ms around 40 weeks PCA, values before 38 weeks PCA are more variable. The overall L-A function gradient is +0.026ms/week (fig. 5.8). This gradient is very slight and would suggest constant data over the term period. The one-sample t test does not show a significant maturation (P>0.05). Wave I has an overall mean value during the term period of 2.17ms (n=28 S.D. 0.309) and a trend value of 2.16ms at 40 weeks PCA. Wave III has a L-A gradient of -0.046ms/week (fig. 5.8) with an overall mean of 5.06ms (n=28 S.D. 0.419) and a predicted value of 5.02ms at 40 weeks PCA.

Wave III shows a more pronounced change with PCA during the term period. The majority of values are below 5.10ms after 38 weeks PCA, there appears to be some latency prolongation before 38 weeks PCA. Wave V displays a more uniform reduction in latency with PCA, the L-A gradient is -0.107ms/week during the term period (fig. 5.8). The overall mean latency for the term period is 7.49ms (n=28 S.D. 0.374), with a trend value of 7.36ms at 40 weeks PCA. Both data for waves III and V fail to show significance (P>0.05) for a maturational trend. This could in part be due to the number of subjects in the term group (n=28).

The wave V L-I function was constructed for all subjects on the study (the two with suspected dysfunction were excluded, see Section 7.2.2). It can be seen that the L-I function decreases with PCA at a rate of -0.0015ms/10dB nHL per week. The overall mean is 0.350ms/10dB nHL (n=26 S.D. 0.150) for the term period.

5.2.1.2 Interpeak Latencies

IPL data was calculated for the I-III, III-V and overall I-V IPL parameters. The behaviour of these parameters was studied by subtraction of the preceding from the latter wave parameter.

The absolute latency data suggests that both waves III and V are affected by GA, with wave I being relatively unaffected. This would lead to the suggestion that a

reduction in latencies would be observed for all IPL parameters. The I-III IPL is the greater portion of the overall I-V IPL with overall mean values of 2.85 (n=27 S.D. 0.361) and 2.46ms (n=27 S.D. 0.270) for the I-III and III-V IPLs respectively. Trend values at 40 weeks PCA are 2.82 and 2.37ms respectively. The I-III IPL has a trend of -0.079ms/week compared to the -0.057ms/week of the III-V IPL (fig. 5.9). These two trends are not significantly different (P>0.05). The I-V IPL has an L-A gradient of -0.124ms/week. This is in line with the reduction of the wave V data (-0.107ms/week) and the increase seen in that of wave I (+0.026ms/week). The trend value at 40 weeks PCA is 5.20ms with an overall mean for the term period of 5.31ms (n=28 S.D. 0.477). As with the absolute latency data. The IPL L-A functions do not reach the required statistical level of probability (P>0.05).

5.2.2 Preterm Data

5.2.2.1 Absolute Latencies

Wave I (13pps) shows a maturational process with an L-A gradient of -0.089ms/week (fig. 5.10, P<0.001). Data is also plotted for mean values in two week periods from 28 to 40 weeks PCA (fig. 5.16), the L-A gradient for these means is -0.102ms/week. The mean reduces from a high at 28-30PCA[c] of 3.57ms (n=4 S.D. 0.745) to a stable value of 2.43ms (n=13 S.D. 0.395) at 38-40PCA (Table 5.8). The 28-30PCA data is non-linear with the later age groups. The high initial mean value is probably due to the reduced number of subjects tested in this age group. These results suggest a maturational process for wave I during the preterm period.

Wave III (13pps) has approximately the same L-A gradient (-0.093ms/week (P<0.001)) as wave I from the raw data (fig. 5.10) and -0.104ms/week for the mean data trend (fig. 5.17). This suggests that the maturation of the I-III IPL is minimal. Means for wave III are more linear, ranging from 6.32ms (n=4 S.D. 0.712) at 28-30PCA to 5.28ms (n=13 S.D. 0.472) at 38-40PCA (Table 5.8).

Wave V (13pps) has a L-A gradient of -0.156ms/week (P<0.001) from the raw data (fig. 5.10) with a -0.186ms/week for the mean trend (fig. 5.18). This suggests maturation of the III-V IPL (waves I and III have the same gradient suggesting a

[c] 28 and 30 refer to 28^{+0} and 30^{+0} weeks PCA.

constant I-III IPL). The reduction in the absolute value of wave I and the convergence of wave V to wave III would suggest that the I-V IPL matures with time. As can be seen, a good level of significance ($P<0.001$ for waves I, III and V) is found for the maturational characteristics of the preterm infants at low stimulus rate. This confirms the tendency for a reduction in ABR latencies at low stimulus rate during the preterm period.

The majority of subjects display L-I gradients between 0.36 and 0.56ms/10dB nHL from 34 weeks PCA onwards. Before 34 weeks more variation is observed. The overall trend is -0.0022ms/10dB nHL per week which does reach the required statistical level of probability ($P>0.05$). Category mean values decrease from 0.528ms/10dB nHL (n=4 S.D. 0.175) at 28-30PCA to 0.458ms/10dB nHL (n=13 S.D. 0.093) at 38-40PCA (Table 5.8). During the period before 32 weeks PCA, the trend is -0.0027ms/10dB nHL per week, from 32 to 36 weeks this falls to -0.0004 (near zero), with a gradient of -0.0014ms/10dB nHL per week from 36 to 40 weeks. The trends for this study suggest a reducing L-I gradient during the preterm period.

5.2.2.2 Interpeak Latencies

Examining the absolute latency trends (13pps), L-A functions give gradients of -0.089, -0.093 and -0.156ms/week for waves I, III and V respectively. This would suggest that the overall trend for the I-III IPL will have a zero gradient. Gradients for the III-V and I-V IPLs should be similar. As gradients for waves I and III are approximately the same, the I-III IPL should be constant with time. A I-III IPL near zero gradient is confirmed from the calculated IPL data (fig. 5.11, L-A gradient +0.005ms/week). The one-sample t test gives a value of $P>0.05$ in comparison with the significant values for the III-V and I-V IPLs. The L-A gradients for the III-V and I-V IPLs, when predicted from latency gradients (ie. wave I or III gradients subtracted from wave V gradient), would give both IPLs an approximate maturational time course of -0.07ms/week. This is confirmed by calculated IPL data, the III-V and I-V IPL gradients are -0.062 ($P<0.001$) and -0.066ms/week ($P<0.005$) respectively (fig. 5.11). The decreasing III-V IPL (constant I-III IPL) causes the I-III/III-V ratio to increase with time.

Mean calculations were made for IPL data, two week categories from 28 to 40 weeks PCA were established (Table 5.8). These support the approximately constant

value of the I-III IPL, means ranging from 3.03ms (n=7 S.D. 0.685) at 30-32PCA to 2.85ms (n=13 S.D. 0.481) at 38-40PCA. The III-V IPL reduces from 3.69 (n=7 S.D. 0.222) to 2.53ms (n=13 S.D. 0.249) and the I-V IPL from 6.44 (n=7 S.D. 0.384) to 5.38ms (n=13 S.D. 0.431) for 28-30PCA and 38-40PCA respectively (figs. 5.19, 5.20 and 5.21). The trends for the mean values are comparable with the other plotting strategies; L-A gradients for the means being -0.0002, -0.082 and -0.084ms/week for I-III, III-V and I-V IPLs respectively.

5.2.3 Term Results Summary (13pps)

- Wave I latency shows a constant trend (at 13pps). The overall mean for wave I is 2.17ms.
- Wave III shows a slight decrease with PCA (≈-0.05ms/week) and displays an overall mean of 5.06ms for the term period.
- Wave V shows the greatest variation with PCA. The L-A function gradient is ≈-0.11ms/week, the overall mean is 7.49ms. However, these maturational trends do not reach the required statistical level of probability (P>0.05).
- The I-V IPL supports the suggestion that the parameters beyond wave I are subject to change during the term period. The L-A function gradient is ≈-0.12ms/week.
- The I-III IPL is greater in latency than the III-V IPL. Means are 2.85 and 2.46ms for the I-III and III-V IPLs respectively. Both regions show slight reductions with PCA during the term period (≈-0.08ms/week for I-III IPL, ≈-0.06ms/week for III-V IPL). These functions do not have significantly different slopes (P>0.05).
- The L-I function gradient shows a slight reduction with PCA during the term period. However, this gradient is slight and cannot be statistically supported (P>0.05).

5.2.4 Preterm Results Summary (13pps)

- All absolute waves (I, III and V) display a reduction at the base stimulus rate, trends for waves I and III have the same L-A gradient, wave V a higher gradient. Reductions are ≈-0.09, ≈-0.09 and ≈-0.16ms/week for waves I, III and V

respectively. All show P<0.001 for the one-sample t test supporting the presence of maturational characteristics during the preterm period.

- The constant I-III IPL suggested from the absolute latency data (waves I and III displaying the same gradients) is confirmed. The I-III IPL data shows a zero L-A function gradient. No statistical significance was found for the slope being different from a zero gradient (P>0.05).
- Both the absolute wave I and the III-V IPL show signs of a reducing latency. The rates are ≈-0.09ms/week (P<0.001) for wave I and ≈-0.06ms/week (P<0.001) for the III-V IPL. The central maturational tendency is supported by the I-V IPL with a L-A function of ≈-0.07ms/week (P<0.005).
- The L-I function is variable before 34 weeks PCA. The overall trend shows a decreasing L-I function with PCA, although the maturational slope does not differ significantly from a zero slope (P>0.05).

5.3 The Rate Effect (37 and 61pps)

It is taken that subtraction of latencies at low stimulus rate (13pps), from higher stimulus rate values, will eliminate the base stimulus rate component. Data plotted in 37-13 and 61-13pps form will, therefore, exclude the base rate component. Rate data is plotted using basic latency data and calculated 37-13 and 61-13pps differences. The L-R function for wave V is also examined.

5.3.1 Term Data

5.3.1.1 Absolute Latencies

The positive L-A gradient for wave I (13pps, +0.026ms/week) is slightly increased when recording at 61pps (fig. 5.8, +0.045ms/week). The 37pps L-A gradient of -0.012ms/week is in disagreement with other stimulus rate data. The validity of this data is questioned due to the 37pps trend suggesting greater values than the 61pps trend at early PCAs. The 37-13 and 61-13pps shift L-A gradients are -0.038 and +0.019ms/week respectively (fig. 5.12, both P>0.05). The overall mean wave I values are 2.43 (n=28 S.D. 0.550) and 2.58ms (n=28 S.D. 0.521) for 37 and 61pps respectively

(13pps, 2.17ms). The means for the 37-13 and 61-13pps shifts are 0.264 (n=28 S.D. 0.382) and 0.414ms (n=28 S.D. 0.376) respectively (Table 5.3). These results suggest that the rate attenuation data for wave I could be unreliable. From the L-A functions for the individual stimulus rates (fig. 5.8) the 61-13pps parameter would seem most valid and shows a relatively constant relationship.

Wave III L-A gradients (fig. 5.8) are -0.104 and -0.083ms/week for 37 and 61pps respectively (13pps, -0.046ms/week). This, again, shows the 37pps data decreasing faster than the 61pps data. However, the 37pps trend remains within the trends for the other stimulus rates. This behaviour causes the 37-13/61-13 ratio to decrease. This is confirmed by the 37-13 and 61-13pps shifts, L-A gradients being -0.058 and -0.037ms/week respectively (fig. 5.12). Overall mean values for the term period from Table 5.3 for 37 and 61pps are 5.45 (n=28 S.D. 0.437) and 5.64ms (n=28 S.D. 0.441) respectively (13pps, 5.06ms). Means for the 37-13 and 61-13pps shifts are 0.388 (S.D. 0.362) and 0.576ms (S.D. 0.346) respectively. This data suggests a reduction of the 61-13pps parameter of ≈-0.04ms/week during the term period. This would lead to the expectation that the I-III IPL will show rate effect maturation during this period.

The most stable results are for the absolute latency of wave V. L-A gradients for 37 and 61pps (fig. 5.8) are -0.133 and -0.150ms/week respectively (13pps, -0.107ms/week). The proportional nature of the decreasing rate effect produces a relatively constant 37-13/61-13 ratio. The increasing L-A gradient with stimulus rate means that the rate effect will become less over time during the term period. The overall means for wave V data at 37 and 61pps are 7.99 (n=28 S.D. 0.492) and 8.33ms (n=28 S.D. 0.503) respectively (13pps, 7.48ms). The 37-13 and 61-13pps shifts have L-A gradients of -0.026 and -0.043ms/week respectively. This shows the same convergence pattern. The mean 37-13 and 61-13pps shifts during the term period are 0.506 (n=28 S.D. 0.330) and 0.846ms (n=28 S.D. 0.307) respectively. The L-A functions at these increased stimulus rates do not achieve the required statistical level of probability ($P>0.05$). Again, this is probably in part due to the number of data points being used.

It would be expected that the L-R function gradient for wave V should decrease in a similar manner as the difference between 13 and 61pps gradients (≈-0.04ms/week). This would result in an L-R gradient decrease of ≈-10μs/decade per week. The relationship of the L-R function with age shows a gradient of -8.98μs/decade per week

(P>0.05), this is in agreement with the decrease suggested by the individual stimulus rate trends. The overall mean L-R function for the term group is 176μs/pps (n=28 S.D. 64), with a trend value of 173μs/pps at 40 weeks PCA. Applying an L-R function to the absolute latency mean data gives a similar result, 177μs/pps (wave V means being 7.48, 7.99 and 8.33ms for 13, 37 and 61pps respectively).

5.3.1.2 Interpeak Latencies

The I-III IPL L-A gradient for 37pps is greater than the 61pps trend, in this case at later PCAs. The 61pps trend does behave well, showing a decreasing I-III IPL rate effect with PCA (61pps, -0.127ms/week; 13pps, -0.079ms/week). The gradient for 37pps is -0.091ms/week. However, neither slopes show a significant difference from zero (P>0.05). The overall mean values are 3.02 (n=28 S.D. 0.385) and 3.05ms (n=28 S.D. 0.431) for 37 and 61pps respectively (13pps, 2.85ms). The 37-13 and 61-13pps shifts for the I-III IPL show gradients of -0.021 and -0.058ms/week (P>0.05) with overall mean values of 0.142 (n=27 S.D. 0.399) and 0.176ms (n=27 S.D. 0.369) respectively. The 37-13/61-13 ratio, thus, increases with PCA reaching a value of 1 at approximately 41 weeks PCA. The suggestion of a I-III IPL maturation from absolute latency data is supported. These results indicate that there is a tendency for the I-III IPL rate attenuation to reduce during the term period (61-13pps rate of ≈-0.06ms/week). Additional data would be required for this to be statistically supported.

The 37pps trend for the III-V IPL also displays a lower gradient than both 13 and 61pps data (-0.029ms/week (P>0.05)). The 61pps data has a slightly higher gradient (-0.068ms/week) than the 13pps trend (-0.057ms/week) and shows a statistically significant maturational slope (P<0.05). Overall mean III-V IPL values are 2.54 (n=28 S.D. 0.315) and 2.69ms (n=28 S.D. 0.327) for 37 and 61pps respectively (13pps, 2.46ms). The reduced 37pps gradient is reflected in the 37-13pps difference, this having a positive trend of +0.029ms/week (mean 0.102ms (n=27 S.D. 0.298)). The 61-13pps shift shows a negligible variation with PCA, the L-A gradient being just -0.005ms/week (fig. 5.13, P>0.05). The 61-13pps mean value is 0.262ms (n=27 S.D. 0.262) for the term group. The 37-13/61-13 ratio again increases with PCA to a value of 1 at 42 weeks PCA. This shows the III-V IPL rate attenuation is relatively constant in the term infant.

To examine the interaction of the I-III and III-V IPLs, the I-III/III-V ratio is a useful tool. With one outlier removed, the I-III/III-V ratio displays trend gradients of -0.021, -0.043 and -0.024 per week for 13, 37 and 61pps respectively (see plots in Appendix D). They all show a decreasing trend with PCA, this supports the previous trend data which suggested that the I-III IPL decreases it's rate attenuation faster than the III-V IPL at all stimulus rates. The difference in rate is not statistically significant ($P>0.05$).

The overall I-V IPL displays a combination of the previous IPL characteristics. The L-A gradients (fig. 5.9) are -0.121 ($P<0.025$) and -0.184ms/week ($P<0.005$) for 37 and 61pps respectively (13pps, -0.133ms/week). The behaviour of the 37pps trend creates an increasing 37-13/61-13 ratio. Overall mean values for the term period are 5.55 (n=28 S.D. 0.439) and 5.77ms (n=28 S.D. 0.469) for 37 and 61pps respectively (13pps, 5.31ms). The 37-13 and 61-13pps shift means are 0.242 (n=28 S.D. 0.453) and 0.456ms (n=28 S.D. 0.413) respectively. Again, the 37-13pps trend is positive (+0.013ms/week) with the 61-13pps trend showing a reduction (fig. 5.13, -0.051ms/week). This supports the tendency for the 61-13pps trend to be more robust than measures at 37pps. It would appear that there is a decreasing rate attenuation for the I-V IPL during the term period.

5.3.2 Preterm Data

5.3.2.1 Absolute Latencies

Considering the absolute latency of wave I, this parameter shows a constant relationship between 13 and 61pps data (fig. 5.10). The relationship between the 13 and 37pps trends increases marginally with time, decreasing the 61-37pps shift. This in turn increases the 37-13/61-13 ratio. The 13 and 61pps relationship seems more robust. The constant relationship between 13 and 61pps data suggests that there is not a rate attenuation maturation present, or that it cannot be detected.

Plotting of the calculated 37-13 and 61-13pps data (for wave I) with time gives positive L-A slopes for both differences (fig. 5.14). The 61-13pps shift trend is +0.057ms/week ($P>0.05$) with the 37-13pps gradient being +0.165ms/week ($P>0.05$). This relationship confirms the tendency to increase the 37-13/61-13 ratio. The lack of a statistical significant difference from a zero slope (with the size of the preterm data)

would indicate a relatively constant rate effect for wave I. These results could be caused by the decreased amplitude and less well defined wave I peaks commonly produced by the preterm population.

Wave III latency trends show that the rate effects for 37 and 61pps are similar in magnitude and gradient (fig. 5.10). The calculated 37-13 and 61-13pps shift trends show a converging pattern. The L-A gradients are -0.014 ($P>0.05$) and -0.050ms/week ($P<0.005$) respectively (fig. 5.14). The 61-13pps data for this current study shows a clear, and statistically supported, reduction with age.

Overall trends for wave V data give L-A gradients of -0.191 ($P<0.001$) and -0.230ms/week ($P<0.001$) for 37 and 61pps respectively (-0.156ms/week for 13pps). These converging trends suggest a reduction in rate attenuation of ≈-0.03 and ≈-0.07ms/week for the 37-13 and 61-13pps shifts respectively. Calculation of these shifts from the raw data gives L-A gradients reducing with time by -0.042 ($P<0.025$) and -0.065ms/week ($P<0.005$) for 37-13 and 61-13pps respectively (fig. 5.14). These trends support the L-A gradients suggested from the basic latency data.

Considering the broader time period trends for the L-R function, both 28-32 and 36-40PCA periods suggest overall increases in L-R gradient, the 32-36 PCA period showing a reduction. Observing the maturation of the L-R function (see plot in Appendix D), it can be seen that the range for 28-30PCA is from zero to nearly 600μs/decade. From the plot it can be seen that L-R gradients before 30 weeks PCA are particularly variable. After 31 weeks PCA a reduction is observed from most subjects through to approximately 34 weeks PCA. However, the suggested positive trend in the 36-40PCA period is actually true for most subjects. This is probably caused by the higher gradient of the 61pps over the 37pps data. There is a suggested convergence at approximately 40 weeks PCA. Being equal, this data (37 and 61pps) would cause lateral movement of the L-R function. This characteristic questions the validity of this function, based as it is, on only three data points. Means for the period show a decline from 30-32PCA onwards, until a slight increase after the 36-38PCA period. The overall trend for the preterm period shows a reduction of -13.44μs/decade ($P<0.001$).

5.3.2.2 Interpeak Latencies

The I-III IPL shows an almost zero gradient at 13pps. However, there is a rate attenuation maturation present (fig. 5.11). The L-A gradients are -0.041 ($P>0.05$) and -0.066ms/week ($P<0.01$) for 37 and 61pps respectively (+0.005ms/week for 13pps). There is a convergence between the 37-13 and 61-13pps shifts with values being equal at 40 weeks PCA. The 61-13pps L-A function shows a gradient of -0.057ms/week ($P<0.005$). These results indicate the presence of a rate attenuation maturation for the I-III IPL.

The III-V IPL shows L-A gradients of -0.096 ($P<0.01$) and -0.088ms/week ($P<0.001$) for 37 and 61pps respectively (-0.062ms/week for 13pps). The trend data suggests a reduction of the 37-13/61-13 ratio with time, the 37pps trend having a higher gradient than the 61pps trend. This relationship is repeated for the calculated 37-13 and 61-13pps shift trends, showing 37-13 declining faster than 61-13pps. L-A gradients are -0.022ms/week for 37-13pps and -0.012ms/week for 61-13pps. Both of these gradients are not significantly different from zero ($P>0.05$). It would appear that the III-V IPL rate attenuation shows a lesser maturation than the process of the I-III IPL. The 61-13pps L-A function gradient is -0.012ms/week for the III-V IPL in comparison to the -0.057ms/week gradient for the I-III IPL.

Considering wave V, with a 61-13pps rate effect of 0.78ms (at 38-40PCA). This is a combination of wave I latency (≈0.3ms) and the delay from the I-V IPL. This suggests a I-V IPL rate effect (61-13pps) of ≈0.5ms (actually 0.49ms from I-V means, 38-40 PCA), the III-V accounting for 0.41ms delay. It is, therefore, suggested that the rate attenuation delay is caused by wave I (≈0.3ms) and the III-V IPL (≈0.5ms). The I-III IPL contributing very little (≈0.07ms).

The I-V IPL data produces a 37-13/61-13 ratio of 0.74ms at 28-30 PCA, this reducing to 0.65ms at 38-40 PCA (Table 5.8). Again, the 37-13pps is more pronounced than the 61-37pps shift. This supports the suggestion that the rate effect is not strictly linear when plotted against stimulus rate. Plotting of the I-V IPL calculated 37-13 and 61-13pps shifts shows that the trends are parallel in nature with L-A gradients of -0.052 ($P<0.05$) and -0.053ms/week (fig. 5.15, $P<0.05$). The parallel nature of these trends is not seen when plotting raw IPL data for the different stimulus rates (fig. 5.11). Overall

trends for the I-V IPL have gradients of -0.132 (P<0.001) and -0.155ms/week (P<0.001) for 37 and 61pps respectively (-0.066 for 13pps). These shifts indicate that the rate effect is more prominent in the parameters beyond wave I.

5.3.3 Term Results Summary (Rate Effect)

- The rate effect on wave I is approximately constant. The rate shift (61-13pps) data shows a slight increasing trend. The overall mean 61-13pps shift for the term group is ≈0.41ms.
- Waves III and V show a decreasing rate effect with PCA. Mean 61-13pps shifts are ≈0.58 and ≈0.85ms for waves III and V respectively. The L-A functions for the 61-13pps shift are both ≈-0.04ms/week. This could suggest a constant rate effect for the III-V IPL.
- The I-III IPL shows an L-A gradient for the 61-13pps shift of ≈-0.06ms/week during the term period. The III-V IPL displays constant data. Overall means for the 61-13pps shift for the I-III and III-V IPLs are ≈0.18 and ≈0.26ms respectively.
- The overall I-V IPL displays a L-A function of ≈-0.05ms/week supporting the behaviour of the I-III and III-V IPLs.
- The L-R function displays a decrease during the term period of ≈-9μs/decade per week with an overall mean value of ≈176μs/decade for the term group.

5.3.4 Preterm Results Summary (Rate Effect)

- The wave I 61-13pps rate attenuation is constant with PCA. Waves III and V show maturational trends of ≈-0.05 (P<0.005) and ≈-0.07ms/week (P<0.005) respectively.
- The clinically used absolute wave V shows a rate attenuation maturation, this being due to the parameters beyond wave I. The central nature of the rate effect maturation is supported by the I-V IPL. The 61-13pps reduction for the I-V IPL is ≈-0.05ms/week (P<0.05).
- The I-III IPL, although constant at the base stimulus rate, displays a maturation in rate attenuation. This occurs (for the 61-13pps shift) at a rate of ≈-0.06ms/week (P<0.005).

- The III-V IPL, however, shows relatively constant rate attenuation with an L-A function of ≈-0.01ms/week. This would be consistent with the lack of difference from a zero slope (P>0.05).
- The trends for rate attenuation maturation suggest that the I-III IPL development is greater than that for the III-V IPL. However, this cannot be supported statistically (P>0.05).
- The L-R function shows an overall reduction with PCA. Values before 30 weeks PCA are extremely variable. In addition, most subjects display a slight increase in the L-R function gradient beyond 36 weeks PCA. This questions the validity of such a parameter. The overall trend suggests a change of ≈-13μs/decade per week.

5.4 Gender Effects

A useful approximation of gender effects on the maturation of preterm infants and the variation with GA in term infants can be gained from the L-A functions for data subdivided for gender. These L-A functions are constructed on the scatter plots contained in Appendices C and D. Numerical data for L-A functions and means for the term group can be seen in Tables 5.2a/b and 5.4a/b respectively. The L-A functions constructed for the female group are considered unreliable due to 79% of these subjects being tested beyond 39 weeks PCA. Function gradients will tend to be dominated by the three infants tested before 39 weeks. The male group shows a more uniform test PCA distribution. A summary of L-A gradients for the preterm infants can be found in Tables 5.7a and 5.7b. Category means (2 week intervals) for gender are reproduced in Tables 5.9a and 5.9b. Care must be taken in analyzing these gender means due to the low number of subjects in some of the categories. This is especially true for the 28-30PCA category where few infants were tested and results tend to be naturally variable.

5.5 Base Stimulus Rate (13pps)

5.5.1 Term Data

Mean values for the term group are presented in Tables 5.4a and 5.4b. Wave I displays a slightly lower overall term period mean for females at 13pps (0.08ms difference). This difference is minor. Wave III also shows no gender effect at 13pps (0.03ms difference). Wave V displays the female mean being 0.12ms lower than that for the male group. These results show similar differences for the base stimulus rate data progressing through the auditory system from wave I to wave V. None of the differences were found to be significant when tested with the unpaired Wilcoxon test. The absolute latency data would not suggest any significant differences for the IPLs.

The L-I function (wave V, 13pps) shows similar mean values for gender, these being 0.452ms/10dB nHL (n=14 S.D. 0.176) for males and 0.391ms/10dB nHL (n=13 S.D. 0.115) for females. This indicates that there is not a clear gender effect ($P>0.05$) for the L-I function.

Gender means for the I-III IPL (13pps) are 2.84 (n=14 S.D. 0.384) and 2.86ms (n=13 S.D. 0.335) for males and females respectively. A similar relationship is found for the overall central region (I-V IPL). The means are 5.34 (n=14 S.D. 0.381) and 5.37ms (n=13 S.D. 0.557) for males and females respectively. These results support the suggestion that the maturation of the I-III IPL shows no gender effect ($P>0.05$) for the term newborn. The I-V IPL parameter suggests that this is also the case for the III-V IPL. The III-V IPL shows only a slight difference in means with values of 2.50 (n=14 S.D. 0.237) and 2.41ms (n=13 S.D. 0.294) for male and female groups respectively. This result was also found not to be significant ($P>0.05$). The IPL data confirms the results suggested by the absolute latency data. It is also noted that the confidence band data in the plots of Appendix C show overlapping confidence bands for gender in all parameters at the base stimulus rate. It is, therefore, proposed that the base stimulus rate data displays no gender difference for the central region in the term infant.

5.5.2 Preterm Data

It is observed that the female group (for this study) generally have lower wave I latency means after 32 weeks PCA for the base stimulus rate (Tables 5.9a and 5.9b). The L-A functions for wave I show similar values and gradients for gender. It is observed in Figure D1b that both gender L-A function confidence bands overlap. With the low subject numbers in the categorized data, the L-A functions are more reliable. This suggests no gender interaction for the base stimulus rate for the latency of wave I. Wave III displays similar mean behaviour for gender between 34 and 38 weeks PCA. The L-A functions show males with a slightly higher gradient. However, the L-A function confidence bands for gender again overlap. This indicates that the data grouped by gender are not from statistically different samples.

Wave V, the commonly used clinical parameter, displays a gender difference with males having prolonged latencies over the entire age range (28-40 weeks PCA). The L-A functions are approximately parallel with males having higher values. However, observing the confidence bands in Figures D7b and D9b, it can be seen that there is not a significant difference between genders at the base stimulus rate. The wave V L-I function (13pps) displays lesser values for females only before 34 weeks PCA. As previously mentioned, the early data is particularly variable. There is not a significant difference beyond 34 weeks PCA. These results would indicate that any possible gender effect would be for wave V latency. In addition, that this gender interaction would be on a central level beyond wave I. In order to study the central system it is necessary to examine the IPL parameters.

The I-V IPL shows an increased L-A gradient for the male group. This causes the gender trends to converge (converged by 40 weeks PCA). The mean data confirms lower values for females between 30 and 38 weeks PCA. The I-III IPL trends show approximately constant values for both male and female groups. There would not appear to be a gender difference for this parameter. This would tend to agree with the absolute latency results suggesting no gender differences for waves I and III. Means are inconsistent over all age categories. Both gender L-A function confidence bands overlap which indicates that they are not significantly different. Mean data for the III-V IPL indicate lesser values for the female group from 32 weeks PCA onwards. The L-A

functions would not support this behaviour, whilst the female trend is higher both L-A function confidence bands overlap.

These results show some evidence of reduced latency values for parameters beyond wave I for the female preterm infant. These differences are not statistically supported. The I-III IPL shows similar base stimulus rate characteristics for both genders. It is, therefore, suggested that any possible gender difference would most likely be due to the properties of the III-V IPL. Female infants have lesser mean values for the III-V IPL from 32 weeks PCA onwards. The difference for the 38-40PCA period is 0.27ms. This behaviour will be discussed further in Chapter Seven in the section on the effects of the preterm birth.

5.5.3 Term Results Summary (Gender)

- There is no base stimulus rate gender effect for the absolute waves I, III and V. None of the minor differences achieved statistically significant levels of probability ($P>0.05$).
- The results for the absolute latency parameters are confirmed by the lack of a gender effect for all IPLs. Again, the unpaired Wilcoxon test found no significant differences ($P>0.05$).
- There is no clear evidence of a gender effect for the wave V L-I function.

5.5.4 Preterm Result Summary (Gender)

- The L-A functions suggest that there is no gender effect for the base stimulus rate data for waves I and III. Wave V mean data and L-A functions both show males having slightly higher values. However, both gender L-A function confidence bands overlap indicating no significant difference.
- Females have lower mean I-V IPLs between 30 and 38 weeks PCA. L-A functions show converging behaviour with the male gradient being higher. However, again both L-A function confidence bands overlap.
- There is no gender effect for the I-III IPL base stimulus rate properties.

- Females have lower mean III-V IPLs from 32 weeks PCA onwards. L-A functions are not significantly different. However, it is suggested that any possible central gender effect would be most likely to occur in the III-V IPL.
- There is no clear gender effect for the L-I function for the preterm period.

5.6 The Rate Effect

5.6.1 Term Data

The slight difference observed at the base stimulus rate is more pronounced at 37 and 61pps, with females having lesser means than males by 0.24 and 0.25ms respectively. However, the differences with the data available are not statistically significant ($P>0.05$). The 61-13pps shift means for the male and female groups are 0.501 (n=14 S.D. 0.355) and 0.326ms (n=14 S.D. 0.377) respectively. Whilst statistical significance cannot be achieved, these results do suggest that the gender difference is more pronounced for the rate effect than base stimulus rate data. The wave III 61-13pps shift means are 0.639 (n=14 S.D. 0.324) and 0.514ms (n=14 S.D. 0.355) for males and females respectively. Again, this shows a greater influence of the rate effect on any possible gender differences. It should be noted that the rate effect gender difference for wave III, although not statistically significant, is larger than that observed for wave I.

Similar behaviour is observed for wave V with the slight difference in favour of the female infants (0.12ms) being increased at higher stimulus rates. This reproduces the characteristics observed with waves I and III. The difference in mean values is 0.31 and 0.40ms for 37 and 61pps in favour of the female group. The 61pps data is significant for a gender difference ($P<0.05$). The lesser rate effect for the female group is confirmed by the 61-13pps difference. This is also statistically supported ($P<0.05$). Male and female means are 0.981 (n=14 S.D. 0.250) and 0.711ms (n=14 S.D. 0.299) respectively. This produces a gender difference in the means (61-13pps) of 0.27ms for this group of infants. This is further supported by the L-R function (wave V) which displays overall means of 204 (S.D. 52) and 148μs/decade (S.D. 62) for males and females respectively. This result also achieves the required level of probability ($P<0.05$). These results show that the rate effect gender difference increases progressing through the auditory system. This eventually allows for a statistically significant difference to be identified for wave V.

All I-III IPL rate data show slightly lower means for the male group. Mean values at 61pps are 2.97 (n=14 S.D. 0.407) and 3.13ms (n=13 S.D. 0.439) for males and females respectively (tables 5.4a and 5.4b). The gender difference for the 61-13pps means is ≈0.08ms in favour of the male group. This is slight and does not achieve the required statistical level of probability ($P>0.05$). The III-V IPL shows a more pronounced gender difference (in favour of the female group), with the 61pps means being 2.85 (n=14 S.D. 0.261) and 2.54ms (n=13 S.D. 0.313) for male and female groups respectively. This difference is statistically significant ($P<0.05$). This relationship is confirmed by the 61-13pps mean shift. The discrepancy between values is ≈0.17ms. The I-V IPL parameter does not show a clear gender difference. The means at 61pps are 5.82 (n=14 S.D. 0.560) and 5.72ms (n=13 S.D. 0.472) for males and females respectively. The female group have approximately the same 61-13pps mean (≈0.05ms difference) as the male group.

These results would suggest that both the I-III IPL and I-V IPL parameters show no gender effect for rate attenuation. There is a slight difference in the 61-13pps parameter for the III-V IPL with the III-V IPL at 61pps being statistically different ($P<0.05$). This would indicate that any possible gender effect for rate attenuation in the term infant would be likely to be located in the III-V IPL region.

5.6.2 Preterm Data

The wave I 61-13pps data displays no clear gender difference at the higher stimulus rates. As with the base stimulus rate data female means are lower after 32 weeks PCA for both 37 and 61pps. However, the L-A functions are very similar in value and gradient with confidence bands overlapping. This is supported by the L-A function characteristics for the 61-13pps parameter. The wave III L-A functions show males with higher values, this difference is exaggerated compared to the behaviour at the base stimulus rate. This behaviour is confirmed by the 61-13pps L-A function which shows males with a consistently higher rate effect.

Wave V also displays a more pronounced gender difference at the higher stimulus rates. The difference in the 61pps mean is 0.71ms at 36-38 PCA. This characteristic is supported by the L-A functions of the 61pps data that display non overlapping

confidence bands. Similar behaviour is noted for the 61-13pps parameter. It is interesting to note that there are no gender differences for means for the 38-40PCA period at all stimulus rates. The 61-13pps trends, however, suggest that the gender difference in the rate effect is greater during the term period than for earlier PCAs. This is supported by the confidence band data for the L-R function (fig. D20b). These results would indicate the presence of an increasing gender difference with rate for the absolute latency parameters.

Examining the I-V IPL rate effect, means for 61pps are consistently lower for the female group. This is also reflected in the 37-13 and 61-13pps mean data, all time periods displaying lower means for the female group (Tables 5.9a and 5.9b). This tendency for a gender difference at higher stimulus rates is supported by the L-A function for 61pps. The 61-13pps data shows the male group having an approximately constant trend, the female group showing a decreasing trend. However, this parameter produces L-A functions with overlapping confidence bands (fig. D30b). Mean data (61-13pps) for the 36-38 and 38-40PCA periods display females being 0.83 and 0.40ms less than those of the male group. These results suggest a tendency for lesser rate attenuation of the parameters beyond wave I in the female preterm infant. However, the L-A function data produced with these results are inconclusive.

The mean data for the I-III IPL (at 37 and 61pps) show no consistent gender difference over the entire preterm period. Means are actually higher for the female group for the 38-40PCA period. L-A functions for data at 37 and 61pps show maturational trends with male gradients being higher. However, the 61-13pps trend suggests a maturational process for the female group with the male rate effect being approximately constant. Mean 61-13pps data shows higher values for females before 34 weeks PCA. Beyond 34 weeks PCA, the female means are lower. However, the 61-13pps means are negative for the female infants beyond 36 weeks PCA. Whilst these means are far less than the male means, the behaviour of the data leads to inconclusive results.

Data for the III-V IPL suggest females to have lower means for 37 and 61pps from 32 weeks PCA onwards. This behaviour is confirmed by L-A functions displaying higher gradients for the female group with lesser values. There is no overlapping of L-A function confidence bands (fig. D15b). The 61-13pps trend also displays a higher gradient for the female group but is not statistically different from that of the male. This

behaviour is supported by mean data for the 37-13 and 61-13pps differences. Means are less for the female group between 30 and 38 weeks PCA. However, these results are contradicted in the 38-40PCA category. The variability of this data does not allow for conclusive gender effects relating to rate attenuation.

These results show some evidence of a central gender effect for the preterm infant. It is likely that any gender effect would be located in the III-V IPL region. This aspect will be further discussed in Chapter Seven where the effects of the preterm birth are examined. It is of interest whether these slight differences in maturation and value lead to significant differences in these preterm subjects by the term period. The unpaired Wilcoxon test will be used to assess the gender interaction on the resultant maturational characteristics during the term period.

5.6.3 Term Results Summary (Rate Effect for Gender)

- The female data appears to show a lesser mean for the 61-13pps rate effect for wave I (difference of ≈0.18ms). However, this result was not found to be significant ($P>0.05$).
- The wave V 61-13pps shows a more pronounced gender difference with the female mean being ≈0.27ms less than the male value. This result is supported by the L-R function data. Statistical significance for gender differences were found for both of these measures ($P<0.05$). The difference for wave V at 61pps was also found to be significant ($P<0.05$).
- The I-III and I-V IPL parameters both show no rate attenuation gender effect.
- The III-V IPL shows a mean 61-13pps shift which is ≈0.17ms lower for the female group. This difference was not found to be significant ($P>0.05$). However, the III-V IPL data at 61pps was found to have a significant gender difference ($P<0.05$).

5.6.4 Preterm Results Summary (Rate Effect for Gender)

- The L-A functions show no gender interaction for the rate effect of wave I. Confidence bands were found to overlap. Wave III L-A functions show males with a consistently higher rate effect during the preterm period.
- There is a suggestion of a central gender effect from the wave V latency data. Differences are noted for the 61pps mean data and L-A function confidence bands were found to be separate. Similar behaviour is found for the 61-13pps parameter.
- The rate shift (61-13pps) means for the I-V IPL are consistently higher for male infants. This supports the suggestion that there could be a central gender effect. L-A functions are however inconclusive.
- The I-III IPL shows no clear gender effect for both mean data and L-A functions. The III-V IPL shows differences in L-A functions and mean data for the 61pps parameter. However, the variability of the 61-13pps data for the I-III and III-V IPLs leads to inconclusive results regarding gender effects on rate attenuation beyond wave I.

5.7 Dietary Analysis

The dietary groupings for this study are >75% volume breast milk (EBM[d]), >75% volume Milupa Prematil formula (containing long chain polyunsaturated fatty acids (LCPUFA)) and >75% volume Cow & Gate (C&G) formula (containing no LCPUFAs)[e]. Dietary intake was measured by volume as precise energy intake calculations for EBM are not possible. Intake was thus expressed by volume with 75% of a particular feed being deemed by the sponsors to be an appropriate target for classification. Of the total 22 preterm infants tested, nineteen fit into these categories. Three infants did not fulfil the criteria of having a diet of >75% volume of any particular feed. These subjects will be discounted from the dietary analysis. There are, therefore, seven infants on breast milk (EBM), seven on Prematil and five on C&G formula.

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[d] Refers to expressed breast milk.

[e] The Cow & Gate formula was specially produced by Cow & Gate Limited for this study.

L-A functions (with statistical data and 95% confidence bands) were constructed for the dietary scatter plots, these can be seen in Appendix D. It is immediately obvious from the absolute latency plots that the C&G infants display L-A gradients not representative of the true maturation pattern of the infants in the group. This calls into question whether the C&G group are a representative sample. Observing the birth statistics for dietary groupings, the EBM and Prematil groups have similar mean GAs, these being 30^{+5} (n=7 S.D. 1^{+4}) and 30^{+4} weeks (n=7 S.D. 1^{+6}) respectively (fig. 5.22). The mean for the C&G group is just 29^{+5} weeks (n=5 S.D. 1^{+5}) (see fig. 5.22). This is reflected in the mean birthweights, these being 1304 (n=7 S.D. 323), 1434 (n=7 S.D. 455) and 1225g (n=5 S.D. 131) for EBM, Prematil and C&G groups respectively (fig. 5.23). The earlier mean GA for the C&G group results in 3 infants from this group being tested during the 29th week PCA (only 1 EBM and no Prematil infants were tested during this week). This situation in combination with an infant tested during the term period with particularly low latency values is sufficient to produce exaggerated L-A gradients which are not representative of individual characteristics. This will limit the usefulness of the L-A functions for the C&G group.

Mean data for the preterm study for the dietary analysis necessitate a broader categorization, this is due to the reduced dietary group numbers. The categories used are 28-32, 32-36 and 36-40[f] weeks PCA. Mean data can be seen in Table 5.10 with plotted data in Figures 5.24 to 5.29. Summary L-A functions can be seen in Figures 5.30 to 5.32. These show information for the I-III, III-V and I-V IPLs at 13 and 61pps. Figures 5.33 to 5.35 show summary L-A functions for the 61-13pps rate shift for these IPLs.

Daily feeding quantities (in ml) were recorded until discharge from the hospital. The majority of formula infants remained on preterm feed until discharge. Apart from two infants, testing was not performed after discharge from the unit. As dietary effects are on a neurological level, the area of interest is the central system beyond the PAS. Absolute latency characteristics for waves I (PAS) and V (clinical use) will be discussed briefly. The discussion of the rate effect will concentrate on the 61-13pps difference, this being the more robust rate parameter.

Statistical analysis was performed on the preterm data collected within the term period (36-40 weeks PCA). This was to assess the effects (by the term period) of the

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[f] These values refer to specific weeks PCA with zero extra days (ie. 32^{+0}).

various dietary schemes. Infants tested during this period are; 7 on EBM, 4 on Prematil, 4 on C&G and 2 undefined (lack of >75% of any one particular diet). Genders are equally split for all groups, apart from the EBM group where there are 2 females and 5 males. The C&G group are not included in this analysis due to the inconsistencies seen in previous analysis. This is probably due to the fact that 3 out of the 4 infants tested during the 29th weeks PCA were assigned to the C&G formula. Also, the 3 C&G infants tested during the term period were in the 36th week PCA, this could introduce an age related effect. Values for the one-sample t test showing significant differences in L-A functions from a zero gradient are presented in the figures of Appendix D. Statistical analysis was performed to compare Prematil and C&G infants to the EBM control group. The unpaired t test was implemented to L-A function slopes. In agreement with the previously stated problem with the C&G group not being representative, many of the L-A functions for this group were found to be significantly different from the EBM control group (P values ranging from <0.05 to <0.005). This supports the behaviour noted from the graphical data. The unpaired Wilcoxon test was performed on dietary grouping data during the term period to assess the effects of diet on the integrity of the preterm auditory system by the term period.

5.7.1 Absolute Latencies (13 and 61pps, 61-13pps)

Considering wave I (13pps), the EBM L-A function suggests lower values than for the Prematil trend but with a lesser L-A gradient (fig. D1c). These trends converge with age. However, the L-A function confidence bands do not overlap. This would suggest a significant difference. The trends at 61pps are similar in value and gradient (with overlapping confidence bands). The relationship at 13pps is supported by the mean data, EBM means being lower than Prematil over the entire period (Table 5.10). Means for EBM, Prematil and C&G during the term period (36-40PCA) are 2.26 (n=10 S.D. 0.251), 2.48 (n=8 S.D. 0.473) and 2.76ms (n=3 S.D. 0.490) respectively (fig. 5.25). There is no significant difference between EBM and Prematil groups (P>0.05). The C&G trends at all stimulus rates are increased due to readings at early PCAs.

The trends for the 61-13pps shift display similar values and gradients for both EBM and Prematil groups (fig. D22c). Again, with overlapping confidence bands. The

C&G group display higher and more variable data with mean values being much higher before 36 weeks PCA. Means for the 61-13pps difference suggest EBM infants have higher values than Prematil after 32 weeks PCA. For the 36-40PCA period, the means for EBM and Prematil groups are 0.42 (n=10 S.D. 0.469) and 0.34ms (n=8 S.D. 0.276) respectively (Table 5.10). The difference in means is 0.08ms. Considering the standard deviation data, the difference observed for the 61-13pps parameter is relatively minor and is not significant (P>0.05).

Wave III shows similar relationships as wave I. The Prematil L-A function is slightly greater at both 13 and 61pps (figs. D4c and D6c), but there is no suggestion of significantly different values (both display overlapping confidence bands). The L-A functions for the 61-13pps difference suggests no difference in value or maturational characteristics (fig. D24c).

The L-A functions for wave V (13pps) also show similar characteristics to those seen for wave I (fig. D7c). The EBM trend has a lesser gradient than that for Prematil, the trends also suggest lower values for the EBM group. Confidence band data supports the difference in values at earlier PCAs with converging behaviour. This is supported by mean values with EBM being consistently lower than Prematil (fig. 5.27). The means during the term period are 7.82 (n=10 S.D. 0.471) and 8.14ms (n=8 S.D. 0.255) for EBM and Prematil respectively. Trends at 61pps show similar behaviour (fig. D9c). The difference in means is greater than those observed for wave I with a discrepancy of 0.32ms during the term period (fig. 5.27). However, the data available does not achieve the required statistical level of probability (P>0.05). The 61-13pps trends show EBM to be approximately constant with Prematil showing a very slight reduction (fig. D26c). The mean values for the 61-13pps shift are approximately the same before 32 weeks PCA. Whilst the mean for Prematil remains constant for the 32-36PCA period, the EBM mean displays a reduction (Table 5.10). This behaviour is reversed for the term period with the Prematil group showing a dramatic reduction giving mean values of 0.65 (n=8 S.D. 0.284) and 0.81ms (n=10 S.D. 0.440) for Prematil and EBM respectively. These characteristics result in similar gradients and values for both the 61-13pps L-A functions (confidence bands overlapping). This behaviour is repeated in the L-R function data. The inconsistent relationship of the rate effect for these groups leads to an inconclusive

result. The C&G trends for latency and the 61-13pps difference are again greatly increased.

The L-I function displays lower means for the EBM group from 32 weeks PCA. The difference between EBM and Prematil infants is relatively constant. The discrepancy in the term period means is ≈0.08ms/10dB nHL (P>0.05).

5.7.2 Interpeak Latencies

5.7.2.1 Base Stimulus Rate (13pps)

L-A functions for the I-V IPL (13pps) are similar in value and gradient for all dietary groups (fig. 5.32). All confidence bands display overlapping. Means for the 32-36PCA period are 5.74 (n=12 S.D. 0.450), 5.82 (n=14 S.D. 0.485) and 6.02ms (n=6 S.D. 0.627) for EBM, Prematil and C&G respectively (fig. 5.29). By the term period (36-40PCA) EBM infants have a mean of 5.56ms (n=10 S.D. 0.483) compared with the Prematil mean of 5.66ms (n=8 S.D. 0.522). The C&G infants show a reduced mean during this period. This suggests that the transmission properties of the central region are the same for EBM and Prematil infants. The unpaired Wilcoxon test shows no significant difference (P>0.05). The reduced C&G mean during this period is unreliable due to the low number of tests.

The I-III IPL L-A function for the overall group is approximately zero (P>0.05). The L-A functions for EBM and Prematil are slight (EBM being positive), the C&G gradient is greater due to early PCA values (fig. 5.30). All show no significant maturational trends (P>0.05) for the one-sample t test and have overlapping confidence bands. Mean values for EBM and Prematil confirm this constant trend with constant means from 32 weeks PCA onwards (fig. 5.27). EBM and Prematil means are 2.95 (n=10 S.D. 0.493) and 2.74ms (n=8 S.D. 0.560) during the term period. These results show some evidence of Prematil infants having a tendency for lower values than EBM infants from 32 weeks PCA. However, the data available does not allow for statistical support. C&G mean values are highest for the period before 36 weeks PCA.

The III-V IPL displays a greater L-A gradient for the EBM infants over the Prematil group (fig. 5.31). The EBM means are consistently lower than Prematil values for all time periods (fig. 5.28). The term period means are 2.62 (n=10 S.D. 0.204) and

2.93ms (n=8 S.D. 0.301) for EBM and Prematil respectively. The C&G L-A function displays a lesser gradient due to low values during the 28-32PCA period. These results show a tendency for EBM infants to have lesser III-V IPLs than the Prematil group during the term period. Again, the difference does not reach the required statistical level of probability (P>0.05).

5.7.2.2 The Rate Effect (61pps)

The rate effect can be observed from latency measures at different stimulus rates and from the latency difference in raw data (61-13pps will be used for this analysis). Considering the I-V IPL L-A functions for 61pps data, there is convergence between high and low (base) rate trends for both EBM and Prematil data (fig. 5.32). The convergence is more dramatic for the Prematil data. This suggests that the data collected for the Prematil infants on this study were more affected by rate, but that their maturation with PCA is greater than the EBM group. The L-A functions for the 61-13pps difference calculated from raw data suggest that the rate effect displayed with the absolute latency data is exaggerated (fig. 5.35). Both EBM and Prematil infants display similar reduction in the rate effect with PCA (overlapping confidence bands). The EBM and Prematil rate attenuation trend values are approximately equal at 40 weeks PCA. Means for EBM, Prematil and C&G for the term period are 0.39 (n=10 S.D. 0.809), 0.32 (n=8 S.D. 0.288) and 0.50ms (n=3 S.D. 0.755) respectively (Table 5.10). The term period means for the latency shift (61-13pps) are similar for EBM and Prematil groups (P>0.05).

Observing the I-III IPL trends for 13 and 61pps, all groups display a convergence between trends, with all crossing during the term period (fig. 5.30). This suggests some error in trend construction. L-A functions for the 61-13pps shift for both EBM and C&G infants display similar functions (confidence bands overlapping), these becoming negative during the term period (fig. 5.33). Both show higher gradients (and lower values) than the Prematil group. This behaviour is confirmed by the 61-13pps means, the Prematil mean for 32-36PCA being elevated. All groups display similar means by the term period. These results suggest there is no detectable dietary interaction on rate attenuation for the I-III IPL. No statistical differences were found between EBM and Prematil groups.

L-A functions for III-V IPL 61pps data suggest a reduction in the rate effect for the Prematil infants (fig. 5.31). The EBM trend, however, displays a slight increase between high and low stimulus rate data. The 61-13pps L-A functions support this behaviour (fig. 5.34). The 61-13pps means display higher values for the Prematil group before 36 weeks PCA. The means during the term period are 0.41 (n=10 S.D. 0.379) and 0.26 (n=8 S.D. 0.357) for EBM and Prematil respectively. This data does not achieve the required statistical level of probability (P>0.05).

5.7.3 Dietary Results Summary

5.7.3.1 Base Stimulus Rate (13pps)

- Wave I data shows significantly different maturational rates at 13pps for the EBM and Prematil groups (confidence bands do not overlap). The EBM trend suggests lower values. This is reflected in a lower mean at term for the EBM infants. The difference observed in means during the term period does not reach the required statistical level of probability (P>0.05).
- Wave III data shows similar behaviour as that of wave I. However, all L-A functions display overlapping confidence bands. This would suggest that these characteristics are not significantly different.
- Wave V data shows convergence of L-A functions, the Prematil group having the higher values. The EBM group show a lesser mean during the term period (0.32ms), although the data available cannot support this statistically.
- The I-V IPL L-A functions are similar in value and gradient for all dietary groups. This is supported during the term period with both EBM and Prematil groups displaying similar means. No significant difference was evident (P>0.05). This would indicate that the conductive properties are the same for both groups in the central region.
- There is no significant maturational trends for the I-III IPL for both EBM and Prematil groups. Mean values show lesser latencies for the Prematil infants. The mean value for the Prematil group is less in the term period (difference of 0.21ms), but was not found to differ significantly (P>0.05).

- Mean data for the III-V IPL shows lesser values for EBM infants throughout the preterm period. This is also the case during the term period where the EBM group mean is 0.31ms less than the Prematil group. This difference did not reach the required statistical level of probability (P>0.05).

5.7.3.2 Rate Effect (61pps)

- The rate effect for the absolute latency parameters tend to be variable and produce inconsistent mean relationships. No statistically significant differences were found (P>0.05).
- L-A functions for the wave I 61pps parameter and the 61-13pps shift show similar gradients and values. No statistically significant differences were evident for the rate effect during the term period (P>0.05).
- Wave V L-A functions also show similar characteristics for both EBM and Prematil infants for the rate effect. Mean data for rate attenuation of wave V shows inconsistent behaviour over time.
- The I-V IPL rate shift (61-13pps) trends are similar for both EBM and Prematil groups suggesting similar maturational characteristics for rate attenuation. The rate attenuation mean for the term period is approximately equal for EBM and Prematil groups. No statistically significant difference was found (P>0.05).
- The I-III IPL rate effect is higher for the Prematil group at earlier PCAs. By the term period all groups display similar 61-13pps shift means. The L-A function for the Prematil group suggests slightly higher values during the preterm period than for the other dietary groups. Mean data suggests no dietary interaction for the I-III IPL by the term period (P>0.05).
- The III-V IPL data shows a rate attenuation maturation for the Prematil group. The EBM group shows a very slight increasing difference between high and low stimulus rates. Whilst 61-13pps means show an increased value for the Prematil group before 36 weeks PCA, the Prematil mean is less than that for the EBM group for the term period. The difference in term period data is not statistically significant (P>0.05).

Table 5.1 L-A function gradients for term group

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	0.026 (28)	-0.012 (28)	0.045 (28)	-0.038 (28)	0.019 (28)
III	-0.046 (28)	-0.104 (28)	-0.083 (28)	-0.058 (28)	-0.037 (28)
V	-0.107 (28)	-0.133 (28)	-0.150 (28)	-0.026 (28)	-0.043 (28)
I-III	-0.079 (27)	-0.091 (28)	-0.127 (28)	-0.021 (27)	-0.058 (27)
III-V	-0.057 (27)	-0.029 (28)	-0.068 (28)	0.029 (27)	-0.005 (27)
I-V	-0.124 (28)	-0.121 (28)	-0.184 (28)	0.013 (28)	-0.051 (28)
I-III/III-V	-0.021 (27)	-0.043 (28)	-0.024 (28)	-	-
L-I *	-0.0015 (26)	-	-	-	-
L-R **	-8.98 (28)	-	-	-	-

*measured in ms/10dB per week
**measured in µs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.2a L-A function gradients for males

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	0.009 (14)	-0.058 (14)	0.023 (14)	-0.068 (14)	0.013 (14)
III	-0.050 (14)	-0.133 (14)	-0.046 (14)	-0.083 (14)	-0.030 (14)
V	-0.085 (14)	-0.138 (14)	-0.128 (14)	-0.053 (14)	-0.043 (14)
I-III	-0.060 (14)	-0.075 (14)	-0.103 (14)	-0.015 (14)	-0.043 (14)
III-V	-0.035 (14)	-0.005 (14)	-0.047 (14)	0.029 (14)	-0.013 (14)
I-V	-0.095 (14)	-0.080 (14)	-0.151 (14)	0.014 (14)	-0.056 (14)
L-I *	-0.0021 (13)	-	-	-	-
L-R **	-8.98 (14)	-	-	-	-

*measured in ms/10dB per week
**measured in µs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.2b L-A function gradients for females

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	0.047 (14)	0.108 (14)	0.125 (14)	0.061 (14)	0.079 (14)
III	0.042 (14)	-0.010 (14)	-0.080 (14)	-0.052 (14)	-0.089 (14)
V	-0.077 (14)	-0.132 (14)	-0.157 (14)	-0.054 (14)	-0.080 (14)
I-III	-0.013 (13)	-0.117 (14)	-0.172 (14)	-0.113 (13)	-0.169 (13)
III-V	-0.114 (13)	-0.122 (14)	-0.111 (14)	-0.006 (13)	0.010 (13)
I-V	-0.114 (14)	-0.239 (14)	-0.265 (14)	-0.115 (14)	-0.141 (14)
L-I *	-0.001 (13)	-	-	-	-
L-R **	-16.70 (14)	-	-	-	-

*measured in ms/10dB per week
**measured in µs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.3 Overall Means (S.D.) for term group

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps	37pps	61pps	37-13pps	61-13pps
I	2.17 (0.309)	2.43 (0.550)	2.58 (0.521)	0.264 (0.382)	0.414 (0.376)
III	5.06 (0.419)	5.45 (0.437)	5.64 (0.441)	0.388 (0.362)	0.576 (0.346)
V	7.49 (0.374)	7.99 (0.492)	8.33 (0.503)	0.506 (0.330)	0.846 (0.307)
I-III	2.85 (0.361)	3.02 (0.385)	3.05 (0.431)	0.142 (0.399)	0.176 (0.369)
III-V	2.46 (0.270)	2.54 (0.315)	2.69 (0.327)	0.102 (0.298)	0.262 (0.314)
I-V	5.31 (0.477)	5.55 (0.439)	5.77 (0.469)	0.242 (0.453)	0.456 (0.413)
L-I *	0.350 (0.150)	-	-	-	-
L-R **	176 (64)	-	-	-	-

Refer to Table 5.1 for number of subjects
*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.4a Overall Means (S.D.) for males

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps	37pps	61pps	37-13pps	61-13pps
I	2.21 (0.338)	2.55 (0.633)	2.71 (0.580)	0.347 (0.397)	0.501 (0.355)
III	5.04 (0.385)	5.45 (0.460)	5.64 (0.426)	0.493 (0.340)	0.639 (0.324)
V	7.55 (0.403)	8.14 (0.455)	8.53 (0.470)	0.591 (0.325)	0.981 (0.250)
I-III	2.84 (0.384)	2.98 (0.365)	2.97 (0.407)	0.146 (0.452)	0.137 (0.366)
III-V	2.50 (0.237)	2.60 (0.223)	2.85 (0.261)	0.099 (0.338)	0.343 (0.333)
I-V	5.34 (0.381)	5.58 (0.366)	5.82 (0.460)	0.244 (0.520)	0.480 (0.450)
L-I *	0.452 (0.176)	-	-	-	-
L-R **	204 (52)	-	-	-	-

Refer to Table 5.2a for number of subjects
*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.4b Overall Means (S.D.) for females

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps	37pps	61pps	37-13pps	61-13pps
I	2.13 (0.271)	2.31 (0.418)	2.46 (0.417)	0.180 (0.348)	0.326 (0.377)
III	5.07 (0.450)	5.36 (0.392)	5.59 (0.452)	0.283 (0.352)	0.514 (0.355)
V	7.43 (0.331)	7.83 (0.480)	8.13 (0.451)	0.420 (0.313)	0.711 (0.299)
I-III	2.86 (0.335)	3.05 (0.401)	3.13 (0.439)	0.138 (0.332)	0.217 (0.367)
III-V	2.41 (0.294)	2.48 (0.376)	2.54 (0.313)	0.106 (0.249)	0.175 (0.267)
I-V	5.37 (0.557)	5.52 (0.500)	5.72 (0.472)	0.240 (0.375)	0.433 (0.371)
L-I *	0.399 (0.115)	-	-	-	-
L-R **	148 (62)	-	-	-	-

Refer to Table 5.2b for number of subjects
*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.5 Predicted 40 week PCA values for term group

Parameter	LATENCY (ms)			Rate Diff. (ms)	
	13pps	37pps	61pps	37-13pps	61-13pps
I	2.16	2.41	2.58	0.25	0.41
III	5.02	5.34	5.55	0.31	0.52
V	7.36	7.88	8.20	0.51	0.83
I-III	2.82	2.93	2.97	0.06	0.10
III-V	2.37	2.54	2.65	0.18	0.32
I-V	5.20	5.46	5.65	0.26	0.45
L-I *	0.415	-	-	-	-
L-R **	173	-	-	-	-

Refer to Table 5.1 for number of subjects

*measured in ms/10dB per week

**measured in μs/decade per week

37-13 and 61-13pps refer to rate differences in raw data

Table 5.6 L-A function gradients for preterm group

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	-0.089 (71)	-0.072 (71)	-0.089 (70)	0.165 (67)	0.057 (67)
III	-0.093 (72)	-0.109 (72)	-0.112 (70)	-0.014 (69)	-0.05 (68)
V	-0.156 (74)	-0.191 (73)	-0.230 (72)	-0.042 (71)	-0.065 (70)
I-III	0.005 (70)	-0.041 (71)	-0.066 (69)	-0.043 (65)	-0.057 (64)
III-V	-0.062 (72)	-0.096 (72)	-0.088 (69)	-0.022 (68)	-0.012 (67)
I-V	-0.066 (71)	-0.132 (71)	-0.155 (69)	-0.052 (65)	-0.053 (65)
L-I *	-0.0022 (74)	-	-	-	-
L-R **	-13.44 (69)	-	-	-	-

*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.7a L-A function gradients for males

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	-0.08 (42)	-0.062 (41)	-0.104 (40)	-0.0001 (39)	-0.0322 (39)
III	-0.119 (41)	-0.123 (42)	-0.188 (40)	-0.0121 (39)	-0.0635 (39)
V	-0.172 (42)	-0.198 (42)	-0.276 (40)	-0.0355 (40)	-0.0364 (39)
I-III	-0.036 (41)	-0.063 (41)	-0.084 (40)	-0.0119 (38)	-0.0028 (38)
III-V	-0.055 (41)	-0.075 (42)	-0.068 (39)	-0.0106 (38)	-0.0126 (38)
I-V	-0.092 (42)	-0.123 (41)	-0.171 (39)	-0.0137 (38)	-0.0129 (38)
L-I *	-0.0137 (42)	-	-	-	-
L-R **	-16.134 (40)	-	-	-	-

*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.7b L-A function gradients for females

Parameter	LATENCY (ms/week)			Rate Diff. (ms/week)	
	13pps (n)	37pps (n)	61pps (n)	37-13pps (n)	61-13pps (n)
I	-0.101 (29)	-0.085 (30)	-0.096 (30)	0.0363 (28)	0.0338 (28)
III	-0.082 (31)	-0.121 (30)	-0.096 (30)	-0.035 (30)	-0.0646 (29)
V	-0.170 (32)	-0.233 (31)	-0.268 (32)	-0.0631 (31)	-0.0986 (31)
I-III	-0.031 (29)	-0.036 (30)	-0.059 (29)	-0.0907 (27)	-0.1128 (26)
III-V	-0.089 (31)	-0.132 (30)	-0.138 (30)	-0.0266 (30)	-0.0303 (29)
I-V	-0.071 (29)	-0.168 (30)	-0.194 (30)	-0.107 (27)	-0.1257 (27)
L-I *	0.0024 (32)	-	-	-	-
L-R **	-20.549 (29)	-	-	-	-

*measured in ms/10dB per week
**measured in μs/decade per week
37-13 and 61-13pps refer to rate differences in raw data

Table 5.8 Category means (S.D.) and n for preterm group

	28-30PCA	30-32PCA	32-34PCA	34-36PCA	36-38PCA	38-40PCA
I (13/s)	3.57 (0.744)4	2.88 (0.489)7	2.77 (0.474)16	2.58 (0.338)19	2.46 (0.410)12	2.43 (0.393)13
I (37/s)	3.77 (1.075)4	3.00 (0.866)6	2.86 (0.584)16	2.87 (0.437)19	2.70 (0.540)13	2.63 (0.354)13
I (61/s)	3.90 (1.142)4	3.45 (0.619)6	3.11 (0.639)16	2.99 (0.575)19	2.78 (0.487)13	2.73 (0.390)13
I (37-13)	0.20 (0.399)4	0.08 (0.452)6	0.16 (0.417)16	0.28 (0.381)19	0.22 (0.417)12	0.23 (0.217)13
I (61-13)	0.33 (0.557)4	0.53 (0.543)6	0.39 (0.532)16	0.33 (0.344)19	0.51 (0.509)12	0.32 (0.354)13
III (13/s)	6.32 (0.712)4	5.91 (0.484)7	5.78 (0.540)16	5.44 (0.494)19	5.34 (0.506)13	5.28 (0.472)13
III (37/s)	7.04 (0.988)4	6.34 (0.547)6	6.1 (0.517)17	5.69 (0.482)19	5.66 (0.821)13	5.56 (0.457)13
III (61/s)	7.43 (1.125)4	6.78 (0.785)6	6.34 (0.746)15	5.97 (0.594)20	5.65 (0.409)12	5.65 (0.397)13
III (37-13)	0.72 (0.363)4	0.4 (0.396)6	0.24 (0.484)16	0.24 (0.329)19	0.23 (0.190)13	0.28 (0.214)13
III (61-13)	1.11 (0.486)4	0.84 (0.610)6	0.57 (0.750)15	0.48 (0.325)19	0.42 (0.161)12	0.37 (0.211)13
V (13/s)	10.01 (0.553)4	8.85 (0.627)7	8.71 (0.555)17	8.42 (0.499)20	8.29 (0.676)13	7.80 (0.481)13
V (37/s)	10.59 (0.792)4	9.76 (0.814)7	9.36 (0.756)17	8.83 (0.639)19	8.70 (1.123)13	8.33 (0.406)13
V (61/s)	11.16 (1.555)4	10.44 (0.960)7	9.80 (1.064)16	9.21 (0.639)20	8.79 (0.549)12	8.59 (0.391)13
V (37-13)	0.59 (0.549)4	0.91 (0.570)7	0.62 (0.361)17	0.45 (0.355)19	0.27 (0.312)13	0.53 (0.230)13
V (61-13)	1.16 (1.197)4	1.59 (0.542)7	0.96 (0.764)16	0.79 (0.369)20	0.65 (0.445)12	0.78 (0.377)13
I-III (13/s)	2.75 (0.422)4	3.03 (0.685)7	2.93 (0.592)15	2.86 (0.386)19	2.88 (0.666)12	2.85 (0.481)13
I-III (37/s)	3.27 (0.605)4	3.34 (0.802)6	3.23 (0.608)16	2.82 (0.378)19	2.96 (0.667)13	2.93 (0.324)13
I-III (61/s)	3.53 (0.728)4	3.33 (0.665)6	3.27 (0.745)15	2.98 (0.416)20	2.87 (0.446)12	2.92 (0.283)12
I-III (37-13)	0.53 (0.226)4	0.32 (0.388)6	0.17 (0.469)15	-0.04 (0.337)19	-0.01 (0.447)12	0.05 (0.371)13
I-III (61-13)	0.78 (0.363)4	0.31 (0.330)6	0.13 (0.549)15	0.15 (0.269)19	0.01 (0.367)12	0.04 (0.417)12
III-V (13/s)	3.69 (0.222)4	2.94 (0.672)7	2.93 (0.455)16	2.94 (0.442)19	2.95 (0.406)13	2.53 (0.249)13
III-V (37/s)	3.56 (0.702)4	3.59 (0.651)6	3.26 (0.431)17	3.14 (0.434)19	3.04 (0.416)13	2.77 (0.262)13
III-V (61/s)	3.54 (1.308)3	3.83 (0.710)6	3.46 (0.551)15	3.24 (0.460)20	3.14 (0.364)12	2.94 (0.341)13
III-V (37-13)	-0.24 (0.533)4	0.54 (0.545)6	0.38 (0.467)16	0.19 (0.482)19	0.05 (0.352)13	0.24 (0.232)13
III-V (61-13)	-0.10 (1.179)3	0.78 (0.281)6	0.59 (0.506)15	0.31 (0.467)19	0.24 (0.428)12	0.41 (0.375)13
I-V (13/s)	6.44 (0.384)4	5.97 (0.828)7	5.92 (0.547)16	5.80 (0.481)19	5.81 (0.862)12	5.38 (0.431)13
I-V (37/s)	6.83 (0.833)4	6.93 (0.953)6	6.42 (0.718)16	5.96 (0.489)19	6.00 (0.907)13	5.70 (0.291)13
I-V (61/s)	7.42 (1.115)3	7.16 (1.001)6	6.69 (0.997)16	6.22 (0.546)20	6.01 (0.558)12	5.87 (0.391)12
I-V (37-13)	0.36 (0.737)4	0.86 (0.461)6	0.40 (0.449)16	0.15 (0.440)19	0.07 (0.596)12	0.27 (0.348)13
I-V (61-13)	0.82 (0.901)3	1.09 (0.503)6	0.59 (0.534)16	0.46 (0.461)19	0.27 (0.647)12	0.47 (0.551)12
L-I (13/s) *	0.528 (0.175)4	0.477 (0.201)7	0.485 (0.181)17	0.474 (0.100)20	0.515 (0.232)13	0.458 (0.093)13
L-R **	241 (249)3	316 (109)7	226 (163)16	163 (77)19	135 (83)11	163 (79)13

* measured in units/week

*measured in ms/10dB

**measured in μs/decade

37-13 and 61-13pps refer to rate differences in raw data

Table 5.9a Category means (S.D.) and n for preterm males

	28-30PCA		30-32PCA		32-34PCA		34-36PCA		36-38PCA		38-40PCA	
	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.
I (13/s)	4.17(2)	0.042	2.58(3)	0.572	2.85(9)	0.472	2.58(13)	0.323	2.63(7)	0.391	2.54(7)	0.420
I (37/s)	4.53(2)	0.297	2.56(3)	0.918	2.93(8)	0.376	3.00(13)	0.408	2.96(7)	0.470	2.67(8)	0.412
I (61/s)	4.59(2)	0.212	3.18(3)	0.681	3.16(8)	0.556	2.99(13)	0.450	3.01(6)	0.327	2.74(8)	0.445
I (37-13)	0.36(2)	0.255	-0.02(3)	0.362	0.20(8)	0.274	0.41(13)	0.275	0.14(7)	0.173	0.14(7)	0.172
I (61-13)	0.42(2)	0.170	0.60(3)	0.730	0.36(8)	0.334	0.41(13)	0.330	0.32(6)	0.173	0.20(7)	0.329
III (13/s)	6.72(2)	0.679	6.18(3)	0.480	5.71(8)	0.562	5.56(13)	0.493	5.51(7)	0.529	5.18(8)	0.451
III (37/s)	7.41(2)	0.976	6.46(3)	0.308	6.09(9)	0.546	5.85(13)	0.494	5.85(7)	1.012	5.54(8)	0.548
III (61/s)	7.83(2)	1.315	6.98(3)	0.601	6.31(8)	0.574	6.12(13)	0.548	5.75(6)	0.199	5.63(8)	0.446
III (37-13)	0.69(2)	0.297	0.28(3)	0.193	0.29(8)	0.191	0.29(13)	0.366	0.14(7)	0.188	0.35(8)	0.171
III (61-13)	1.11(2)	0.636	0.80(3)	0.524	0.67(8)	0.911	0.56(13)	0.327	0.42(6)	0.131	0.44(8)	0.128
V (13/s)	10.32(2)	0.339	8.92(3)	0.466	8.72(9)	0.604	8.53(13)	0.493	8.45(7)	0.852	7.82(8)	0.541
V (37/s)	10.68(2)	0.085	9.90(3)	0.681	9.48(9)	0.809	9.09(13)	0.564	9.12(7)	1.377	8.35(8)	0.470
V (61/s)	11.31(2)	0.127	10.62(3)	0.888	9.98(8)	1.389	9.41(13)	0.582	9.14(6)	0.251	8.62(8)	0.403
V (37-13)	0.36(2)	0.424	0.98(3)	0.774	0.72(9)	0.270	0.56(13)	0.356	0.44(7)	0.358	0.53(8)	0.221
V (61-13)	0.99(2)	0.212	1.70(3)	0.434	1.02(8)	0.222	0.88(13)	0.346	0.97(6)	0.357	0.80(8)	0.449
I-III (13/s)	2.55(2)	0.636	3.60(3)	0.312	2.82(8)	0.345	2.98(13)	0.385	2.88(7)	0.715	2.65(8)	0.343
I-III (37/s)	2.88(2)	0.679	3.90(3)	0.780	3.15(8)	0.636	2.85(13)	0.394	2.89(7)	0.734	2.87(8)	0.395
I-III (61/s)	3.24(2)	1.123	3.80(3)	0.227	3.15(8)	0.801	3.13(13)	0.409	2.74(6)	0.223	2.89(8)	0.320
I-III (37-13)	0.33(2)	0.042	0.30(3)	0.533	0.10(8)	0.248	-0.12(13)	0.335	0.00(7)	0.240	0.22(8)	0.294
I-III (61-13)	0.69(2)	0.407	0.20(3)	0.308	-0.02(8)	0.377	0.15(13)	0.207	0.10(8)	0.168	0.24(8)	0.316
III-V (13/s)	3.60(2)	0.339	2.74(3)	0.874	3.02(8)	0.474	2.97(13)	0.479	2.94(7)	0.546	2.63(8)	0.171
III-V (37/s)	3.27(2)	1.061	3.44(3)	0.970	3.39(9)	0.470	3.24(13)	0.480	3.27(7)	0.410	2.81(8)	0.219
III-V (61/s)	2.64(1)	-	3.64(3)	1.051	3.68(8)	0.614	3.29(13)	0.535	3.39(6)	0.315	2.99(8)	0.391
III-V (37-13)	-0.84(1)	-	0.70(3)	0.815	0.44(8)	0.295	0.28(13)	0.529	0.30(7)	0.329	0.18(8)	0.257
III-V (61-13)	-0.72(1)	-	0.90(3)	0.393	0.75(8)	0.465	0.32(13)	0.499	0.55(6)	0.401	0.36(8)	0.424
I-V (13/s)	6.15(2)	0.297	6.34(3)	1.033	5.87(9)	0.477	5.94(13)	0.396	5.82(7)	1.080	5.28(8)	0.419
I-V (37/s)	6.15(2)	0.382	7.34(3)	1.245	6.41(8)	0.788	6.10(13)	0.451	6.16(7)	1.092	5.68(8)	0.264
I-V (61/s)	6.66(1)	-	7.44(3)	1.264	6.83(8)	1.301	6.42(13)	0.524	6.13(6)	0.378	5.88(8)	0.385
I-V (37-13)	-0.48(1)	-	1.00(3)	0.618	0.44(8)	0.297	0.15(13)	0.405	0.30(7)	0.459	0.40(8)	0.325
I-V (61-13)	0.30(1)	-	1.10(3)	0.701	0.66(8)	0.367	0.48(13)	0.481	0.65(6)	0.462	0.60(8)	0.557
L-I (13/s) *	0.533(2)	0.092	0.62(3)	0.221	0.536(9)	0.182	0.448(13)	0.093	0.575(7)	0.305	0.454(8)	0.105
L-R **	206(2)	44	320(3)	83	260(8)	223	181(13)	73	202(6)	74	167(8)	94

*measured in ms/10dB

**measured in μs/decade

37-13 and 61-13pps refer to rate differences in raw data

Standard deviations marked '-' indicate single subject data

Table 5.9b Category means (S.D.) and n for preterm females

	28-30PCA		30-32PCA		32-34PCA		34-36PCA		36-38PCA		38-40PCA	
	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.	Mean(n)	S.D.
I (13/s)	2.97(2)	0.467	3.11(4)	0.572	2.67(7)	0.472	2.56(6)	0.323	2.22(5)	0.39	2.26(5)	0.42
I (37/s)	3.00(2)	1.018	3.44(3)	0.918	2.79(8)	0.376	2.61(6)	0.408	2.39(6)	0.47	2.56(5)	0.412
I (61/s)	3.21(2)	1.400	3.72(3)	0.681	3.06(8)	0.556	3.00(7)	0.450	2.55(6)	0.33	2.72(4)	0.445
I (37-13)	0.03(2)	0.552	0.18(3)	0.362	0.12(7)	0.274	0.04(6)	0.275	0.32(5)	0.17	0.41(5)	0.172
I (61-13)	0.24(2)	0.933	0.46(3)	0.730	0.41(7)	0.334	0.17(6)	0.330	0.70(5)	0.17	0.54(4)	0.329
III (13/s)	5.91(2)	0.636	5.72(4)	0.480	5.85(8)	0.562	5.17(6)	0.493	5.13(6)	0.53	5.42(5)	0.451
III (37/s)	6.66(2)	1.189	6.22(3)	0.308	6.11(8)	0.546	5.35(6)	0.494	5.44(6)	1.01	5.59(5)	0.548
III (61/s)	7.02(2)	1.188	6.58(3)	0.601	6.39(7)	0.574	5.69(7)	0.548	5.54(6)	0.20	5.69(5)	0.446
III (37-13)	0.75(2)	0.552	0.52(3)	0.793	0.18(8)	0.191	0.15(6)	0.366	0.31(6)	0.19	0.17(5)	0.171
III (61-13)	1.11(2)	0.552	0.88(3)	0.524	0.48(7)	0.911	0.30(6)	0.327	0.41(6)	0.13	0.26(5)	0.128
V (13/s)	9.69(2)	0.636	8.81(4)	0.466	8.69(8)	0.604	8.2(7)	0.493	8.10(6)	0.85	7.79(5)	0.541
V (37/s)	10.50(2)	1.358	9.66(4)	0.681	9.22(8)	0.809	8.26(6)	0.564	8.20(6)	1.38	8.30(5)	0.47
V (61/s)	11.01(2)	2.673	10.31(4)	0.888	9.61(8)	1.389	8.84(7)	0.582	8.43(6)	0.25	8.54(5)	0.403
V (37-13)	0.81(2)	0.721	0.86(4)	0.774	0.53(8)	0.270	0.20(6)	0.356	0.10(6)	0.36	0.52(5)	0.221
V (61-13)	1.32(2)	2.036	1.50(4)	0.434	0.92(8)	0.222	0.59(7)	0.346	0.33(6)	0.36	0.76(5)	0.449
I-III (13/s)	2.94(2)	0.170	2.61(4)	0.312	3.05(7)	0.345	2.61(6)	0.385	2.88(5)	0.72	3.17(5)	0.343
I-III (37/s)	3.66(2)	0.170	2.78(3)	0.780	3.32(8)	0.636	2.74(6)	0.394	3.05(6)	0.73	3.04(5)	0.395
I-III (61/s)	3.81(2)	0.212	2.86(3)	0.227	3.40(7)	0.801	2.69(7)	0.409	2.99(6)	0.22	2.99(4)	0.32
I-III (37-13)	0.72(2)	0.000	0.34(3)	0.533	0.23(7)	0.248	0.11(6)	0.335	-0.01(5)	0.24	-0.29(5)	0.294
I-III (61-13)	0.87(2)	0.382	0.42(3)	0.308	0.28(7)	0.377	0.13(6)	0.207	-0.10(5)	0.17	-0.36(4)	0.316
III-V (13/s)	3.78(2)	0.000	3.09(4)	0.874	2.84(8)	0.474	2.89(6)	0.479	2.97(6)	0.55	2.36(5)	0.171
III-V (37/s)	3.84(2)	0.170	3.74(3)	0.970	3.11(8)	0.470	2.91(6)	0.480	2.76(6)	0.41	2.71(5)	0.219
III-V (61/s)	3.99(2)	1.485	4.02(3)	1.050	3.22(7)	0.614	3.15(7)	0.535	2.89(6)	0.32	2.86(5)	0.391
III-V (37-13)	0.06(2)	0.170	0.38(3)	0.815	0.33(8)	0.295	0.02(6)	0.529	-0.21(6)	0.33	0.35(5)	0.257
III-V (61-13)	0.21(2)	1.485	0.66(3)	0.393	0.42(7)	0.465	0.29(6)	0.499	-0.08(6)	0.40	0.49(5)	0.424
I-V (13/s)	6.72(2)	0.170	5.70(4)	1.033	5.97(7)	0.477	5.50(6)	0.396	5.80(5)	1.08	5.53(5)	0.419
I-V (37/s)	7.50(2)	0.339	6.52(3)	1.245	6.43(8)	0.788	5.65(6)	0.451	5.81(6)	1.09	5.75(5)	0.264
I-V (61/s)	7.80(2)	1.273	6.88(3)	1.264	6.55(8)	1.301	5.84(7)	0.524	5.88(6)	0.38	5.84(4)	0.385
I-V (37-13)	0.78(2)	0.170	0.72(3)	0.618	0.37(7)	0.297	0.15(6)	0.405	-0.22(5)	0.46	0.02(5)	0.325
I-V (61-13)	1.08(2)	1.103	1.08(3)	0.701	0.51(7)	0.367	0.42(6)	0.481	-0.18(5)	0.46	0.20(4)	0.557
L-I (13/s) *	0.522(2)	0.288	0.369(4)	0.221	0.428(8)	0.182	0.521(7)	0.093	0.446(6)	0.305	0.463(5)	0.105
L-R **	275(2)	424	312(4)	83	190(8)	223	122(6)	73	68(5)	74	157(5)	94

*measured in ms/10dB

**measured in μs/decade

37-13 and 61-13pps refer to rate differences in raw data

Table 5.10 Broad category means (n) for dietary groups

Standard deviations can be found in following table on next page.

Parameter	LATENCY (ms)								
	28-32PCA			32-36PCA			36-40PCA		
	EBM(n)	Prematil(n)	C&G(n)	EBM(n)	Prematil(n)	C&G(n)	EBM(n)	Prematil(n)	C&G(n)
I (13/s)	2.72(3)	3.18(1)	3.41(6)	2.58(12)	2.79(14)	2.63(8)	2.26(10)	2.48(8)	2.76(3)
I (37/s)	2.48(3)	3.18(1)	3.74(6)	2.87(12)	2.78(13)	3.06(8)	2.56(10)	2.64(8)	2.88(3)
I (61/s)	2.70(3)	3.36(1)	4.14(6)	3.08(13)	2.90(13)	3.30(8)	2.68(10)	2.81(8)	3.02(3)
I (37-13)	-0.24(3)	0.00(1)	0.33(6)	0.26(12)	0.04(13)	0.43(8)	0.30(10)	0.17(8)	0.12(3)
I (61-13)	-0.02(3)	0.18(1)	0.73(6)	0.38(12)	0.13(13)	0.67(8)	0.42(10)	0.34(8)	0.26(3)
III (13/s)	5.42(3)	6.12(1)	6.42(6)	5.51(12)	5.57(13)	5.68(8)	5.21(8)	5.21(8)	5.22(3)
III (37/s)	5.80(3)	6.18(1)	7.10(6)	5.70(12)	5.95(14)	6.08(8)	5.42(8)	5.51(8)	5.32(3)
III (61/s)	6.06(3)	6.42(1)	7.63(6)	5.94(13)	6.20(12)	6.45(8)	5.60(8)	5.60(8)	5.54(3)
III (37-13)	0.38(3)	0.06(1)	0.68(6)	0.18(12)	0.28(13)	0.40(8)	0.22(8)	0.29(8)	0.10(3)
III (61-13)	0.64(3)	0.30(1)	1.21(6)	0.34(12)	0.67(12)	0.77(8)	0.40(8)	0.39(8)	0.32(3)
V (13/s)	8.84(3)	9.66(1)	9.63(6)	8.38(13)	8.61(14)	8.66(8)	7.82(8)	8.14(8)	7.80(3)
V (37/s)	9.72(3)	10.08(1)	10.45(6)	8.80(12)	9.30(14)	9.06(8)	8.25(8)	8.45(8)	8.24(3)
V (61/s)	9.86(3)	10.62(1)	11.35(6)	9.18(13)	9.71(13)	9.56(8)	8.63(8)	8.79(8)	8.56(3)
V (37-13)	0.88(3)	0.42(1)	0.82(6)	0.48(12)	0.66(14)	0.41(8)	0.43(8)	0.31(8)	0.44(3)
V (61-13)	1.02(3)	0.96(1)	1.72(6)	0.80(13)	0.95(13)	0.91(8)	0.81(8)	0.65(8)	0.76(3)
I-III (13/s)	2.70(3)	2.94(1)	3.01(6)	2.93(12)	2.75(13)	3.05(8)	2.95(10)	2.74(8)	2.46(3)
I-III (37/s)	3.32(3)	3.00(1)	3.36(6)	2.83(12)	3.16(13)	3.02(8)	2.86(10)	2.87(8)	2.44(3)
I-III (61/s)	3.36(3)	3.06(1)	3.49(6)	2.85(13)	3.35(12)	3.15(8)	2.92(10)	2.79(8)	2.52(3)
I-III (37-13)	0.62(3)	0.06(1)	0.35(6)	-0.09(12)	0.27(13)	-0.03(8)	-0.08(10)	0.13(8)	-0.02(3)
I-III (61-13)	0.06(3)	0.12(1)	0.48(6)	-0.04(12)	0.41(12)	0.11(8)	-0.02(10)	0.05(8)	0.06(3)
III-V (13/s)	3.42(3)	3.54(1)	3.21(6)	2.82(12)	3.04(13)	2.98(8)	2.62(10)	2.93(8)	2.58(3)
III-V (37/s)	3.92(3)	3.90(1)	3.35(6)	3.11(12)	3.35(14)	2.99(8)	2.83(10)	2.94(8)	2.92(3)
III-V (61/s)	3.80(3)	4.20(1)	3.60(5)	3.24(13)	3.53(12)	3.11(8)	3.03(10)	3.19(8)	3.02(3)
III-V (37-13)	0.50(3)	0.36(1)	0.13(6)	0.27(12)	0.37(13)	0.01(8)	0.21(10)	0.02(8)	0.34(3)
III-V (61-13)	0.38(3)	0.66(1)	0.52(5)	0.46(12)	0.53(12)	0.14(8)	0.41(10)	0.26(8)	0.44(3)
I-V (13/s)	6.12(3)	6.48(1)	6.22(6)	5.74(12)	5.82(14)	6.02(8)	5.56(10)	5.66(8)	5.04(3)
I-V (37/s)	7.24(3)	6.90(1)	6.71(6)	5.94(12)	6.42(13)	6.00(8)	5.69(10)	5.81(8)	5.36(3)
I-V (61/s)	7.16(3)	7.26(1)	7.30(5)	6.10(13)	6.81(13)	6.26(8)	5.95(10)	5.98(8)	5.54(3)
I-V (37-13)	1.12(3)	0.42(1)	0.49(6)	0.20(12)	0.56(13)	-0.02(8)	0.13(10)	0.14(8)	0.32(3)
I-V (61-13)	1.04(3)	0.78(1)	1.02(5)	0.42(12)	0.83(13)	0.24(8)	0.39(10)	0.32(8)	0.50(3)
L-I (13/s) *	0.623(3)	0.322(1)	0.503(6)	0.466(13)	0.542(14)	0.431(8)	0.430(10)	0.514(8)	0.476(3)
L-R **	212(2)	200(1)	341(6)	165(12)	244(13)	160(8)	168(10)	135(8)	158(2)

*measured in ms/10dB

**measured in μs/decade

37-13 and 61-13pps refer to rate differences in raw data

Table 5.10 (Cont.) Broad category Standard Deviations (S.D.) for dietary groups

Parameter	LATENCY (ms)								
	28-32PCA			32-36PCA			36-40PCA		
	EBM	Prematil	C&G	EBM	Prematil	C&G	EBM	Prematil	C&G
I (13/s)	0.485	-	0.628	0.365	0.269	0.628	0.238	0.441	0.591
I (37/s)	0.399	-	1.010	0.367	0.492	0.726	0.233	0.425	0.523
I (61/s)	0.661	-	0.488	0.611	0.560	0.157	0.311	0.368	0.592
I (37-13)	0.159	-	0.403	0.228	0.490	0.392	0.418	0.231	0.104
I (61-13)	0.352	-	0.458	0.271	0.449	0.446	0.469	0.276	0.211
III (13/s)	0.302	-	0.459	0.551	0.442	0.656	0.440	0.355	0.591
III (37/s)	0.330	-	0.595	0.401	0.558	0.628	0.352	0.368	0.567
III (61/s)	0.375	-	0.670	0.613	0.802	0.693	0.349	0.322	0.745
III (37-13)	0.035	-	0.447	0.302	0.305	0.502	0.168	0.204	0.271
III (61-13)	0.092	-	0.567	0.293	0.667	0.422	0.179	0.222	0.250
V (13/s)	0.544	-	0.782	0.531	0.606	0.467	0.488	0.456	0.300
V (37/s)	0.884	-	0.785	0.505	0.981	0.721	0.269	0.425	0.781
V (61/s)	1.033	-	1.065	0.531	1.230	0.751	0.348	0.580	0.972
V (37-13)	0.674	-	0.638	0.216	0.379	0.495	0.270	0.267	0.481
V (61-13)	1.062	-	0.780	0.162	0.375	0.466	0.440	0.284	0.716
I-III (13/s)	0.787	-	0.654	0.457	0.384	0.660	0.574	0.475	0
I-III (37/s)	0.716	-	0.799	0.495	0.606	0.504	0.243	0.489	0.183
I-III (61/s)	1.039	-	0.558	0.533	0.696	0.452	0.172	0.330	0.159
I-III (37-13)	0.125	-	0.375	0.317	0.484	0.329	0.493	0.261	0.183
I-III (61-13)	0.433	-	0.417	0.216	0.465	0.372	0.522	0.225	0.159
III-V (13/s)	0.344	-	0.769	0.467	0.494	0.358	0.212	0.382	0.433
III-V (37/s)	0.567	-	0.662	0.311	0.551	0.200	0.205	0.314	0.277
III-V (61/s)	0.870	-	1.033	0.387	0.681	0.289	0.323	0.405	0.227
III-V (37-13)	0.642	-	0.739	0.420	0.394	0.461	0.245	0.281	0.454
III-V (61-13)	1.107	-	0.743	0.351	0.509	0.521	0.379	0.357	0.601
I-V (13/s)	0.991	-	0.696	0.450	0.485	0.627	0.625	0.402	0.433
I-V (37/s)	1.230	-	0.753	0.601	0.677	0.533	0.235	0.379	0.454
I-V (61/s)	1.369	-	0.981	0.620	1.048	0.367	0.348	0.477	0.381
I-V (37-13)	0.517	-	0.567	0.270	0.356	0.582	0.646	0.299	0.500
I-V (61-13)	0.783	-	0.661	0.288	0.459	0.679	0.809	0.288	0.755
L-I (13/s) *	0.122	-	0.183	0.100	0.151	0.131	0.075	0.128	0.098
L-R **	221	-	163	34	183	94	92	49	149

*measured in ms/10dB

**measured in μs/decade

37-13 and 61-13pps refer to rate differences in raw data

Standard deviations marked '-' indicate single subject data

Figure 5.1 Boxplot - Gender versus GA for term group

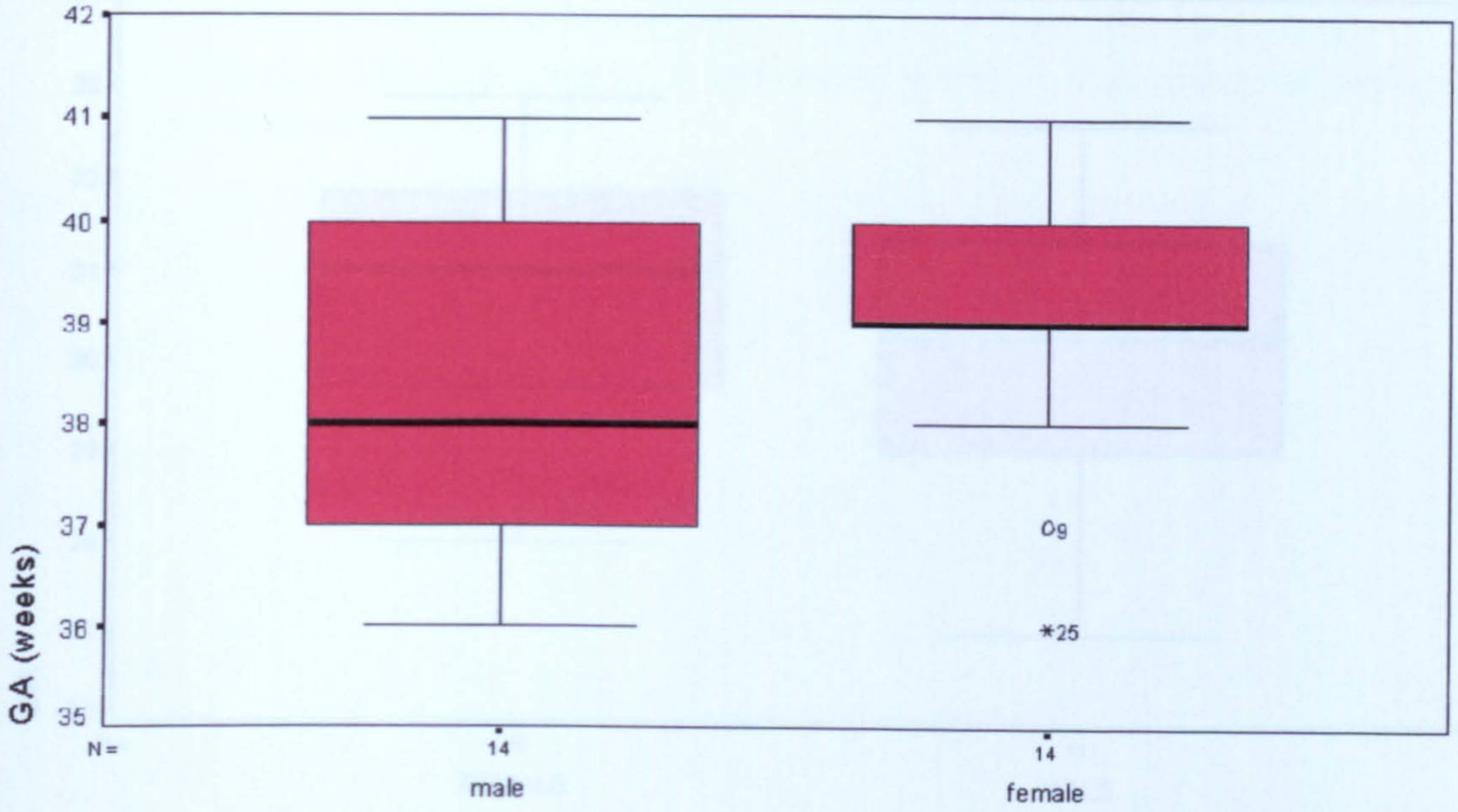
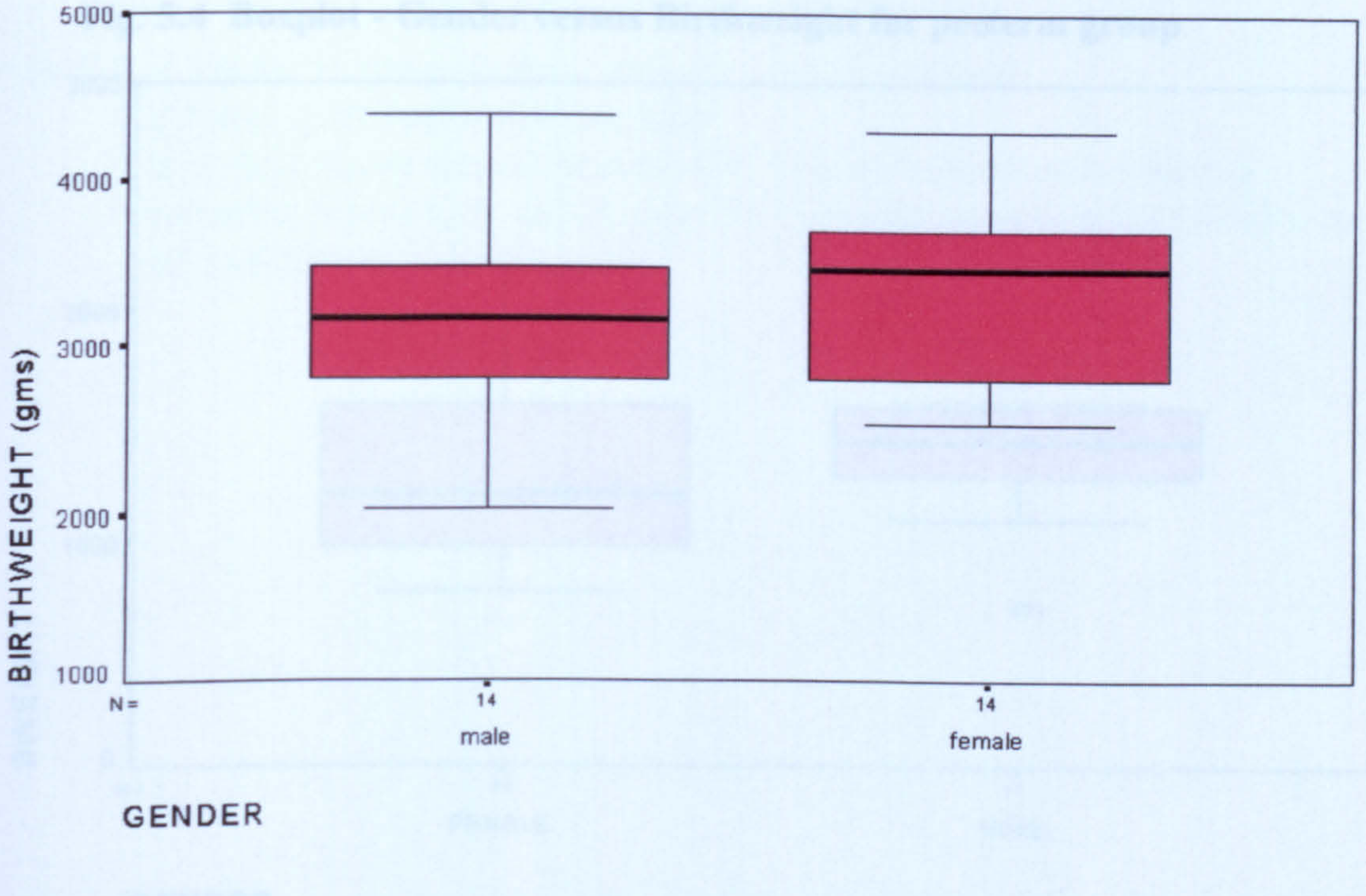


Figure 5.2 Boxplot - Gender versus Birthweight for term group



"Boxes" contain values between the 25th and 75th percentiles, the "whiskers" extend to the highest and lowest values, excluding outliers. A line across the box indicates the median. Any outliers present are plotted separately.

Fig. 5.3 Boxplot - Gender versus GA for preterm group

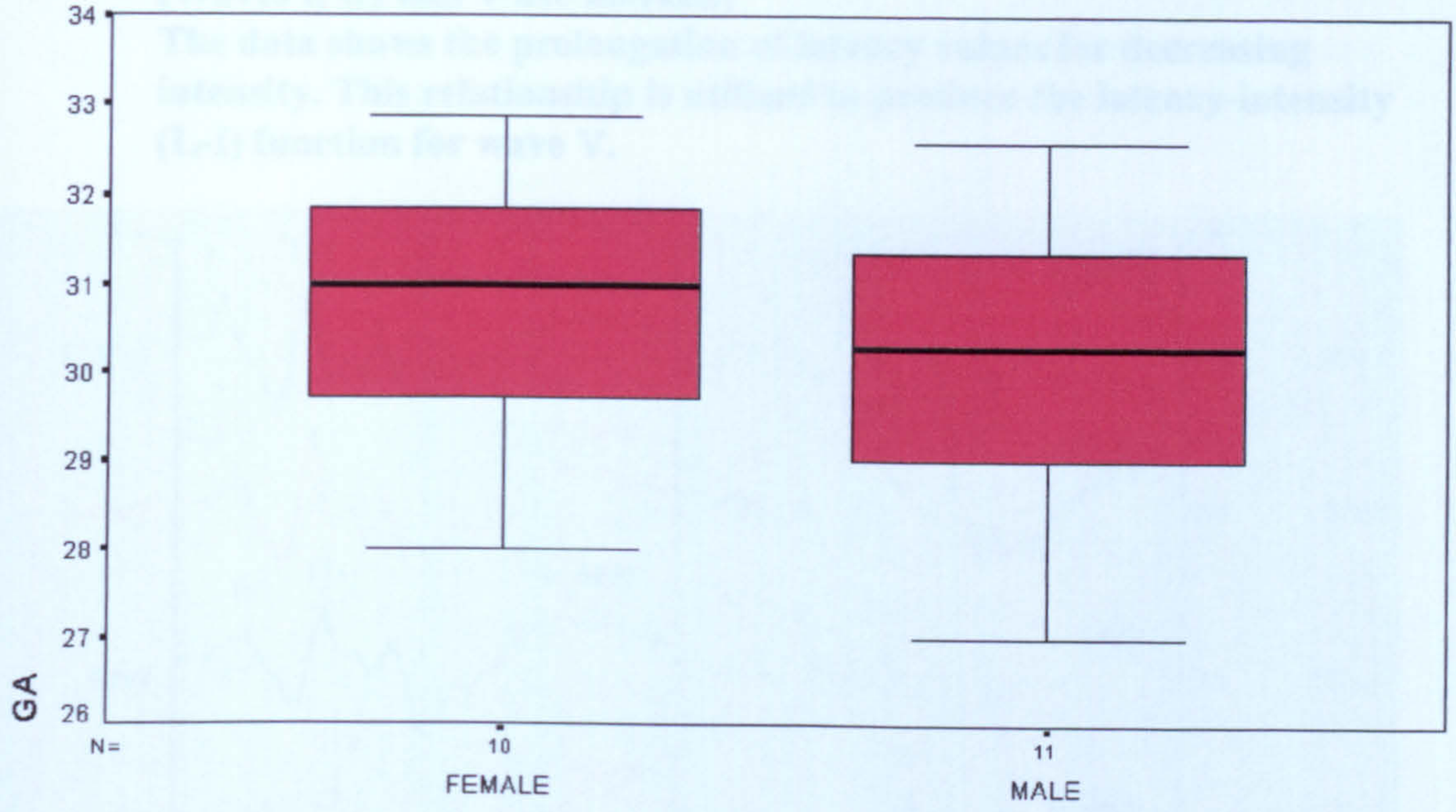
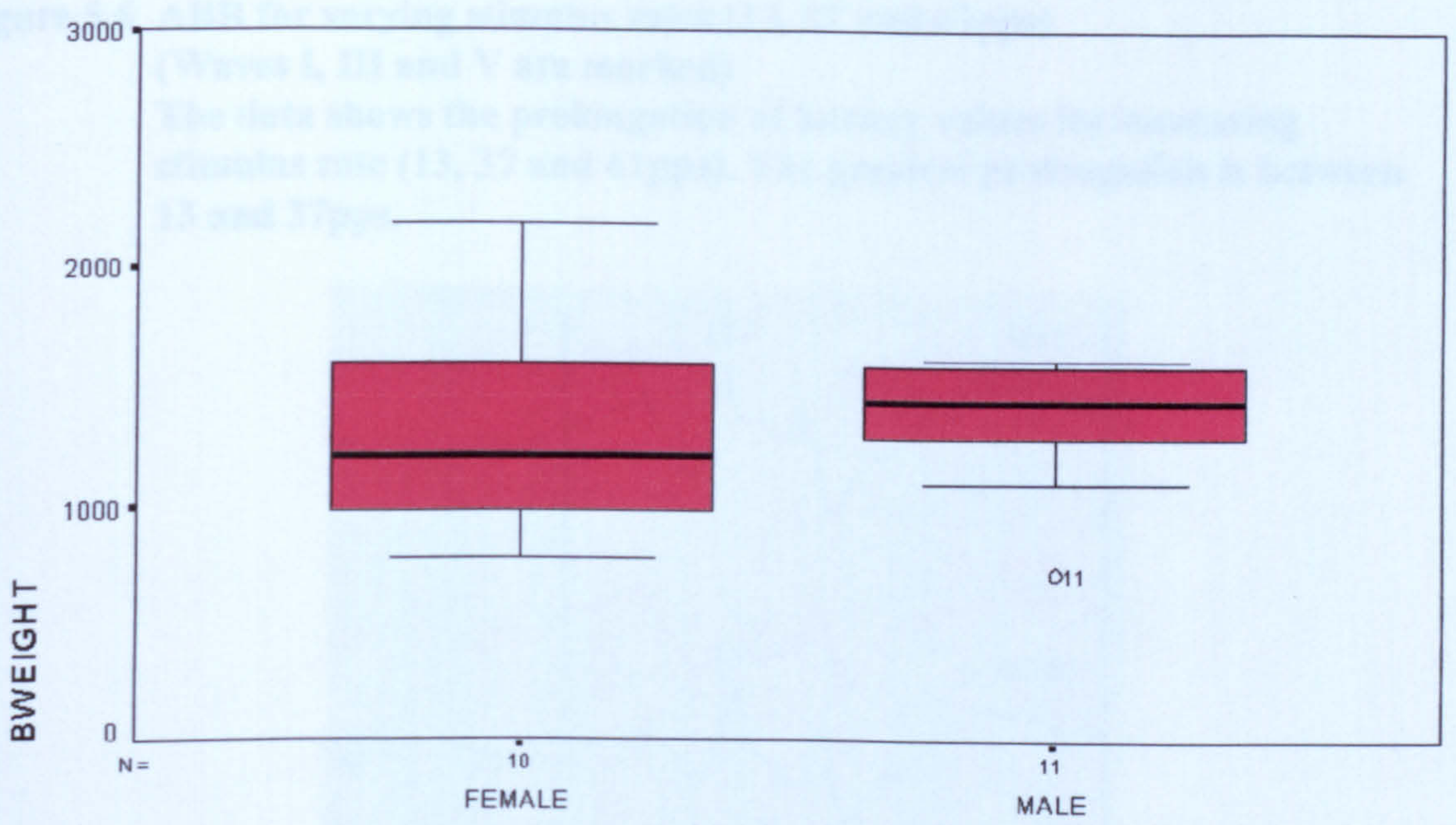


Fig. 5.4 Boxplot - Gender versus Birthweight for preterm group



"Boxes" contain values between the 25th and 75th percentiles, the "whiskers" extend to the highest and lowest values, excluding outliers. A line across the box indicates the median. Any outliers present are plotted separately.

Figure 5.5 ABR for intensity range of 10 to 80dB (Waves I, III and V are marked) The data shows the prolongation of latency values for decreasing intensity. This relationship is utilized to produce the latency-intensity (L-I) function for wave V.

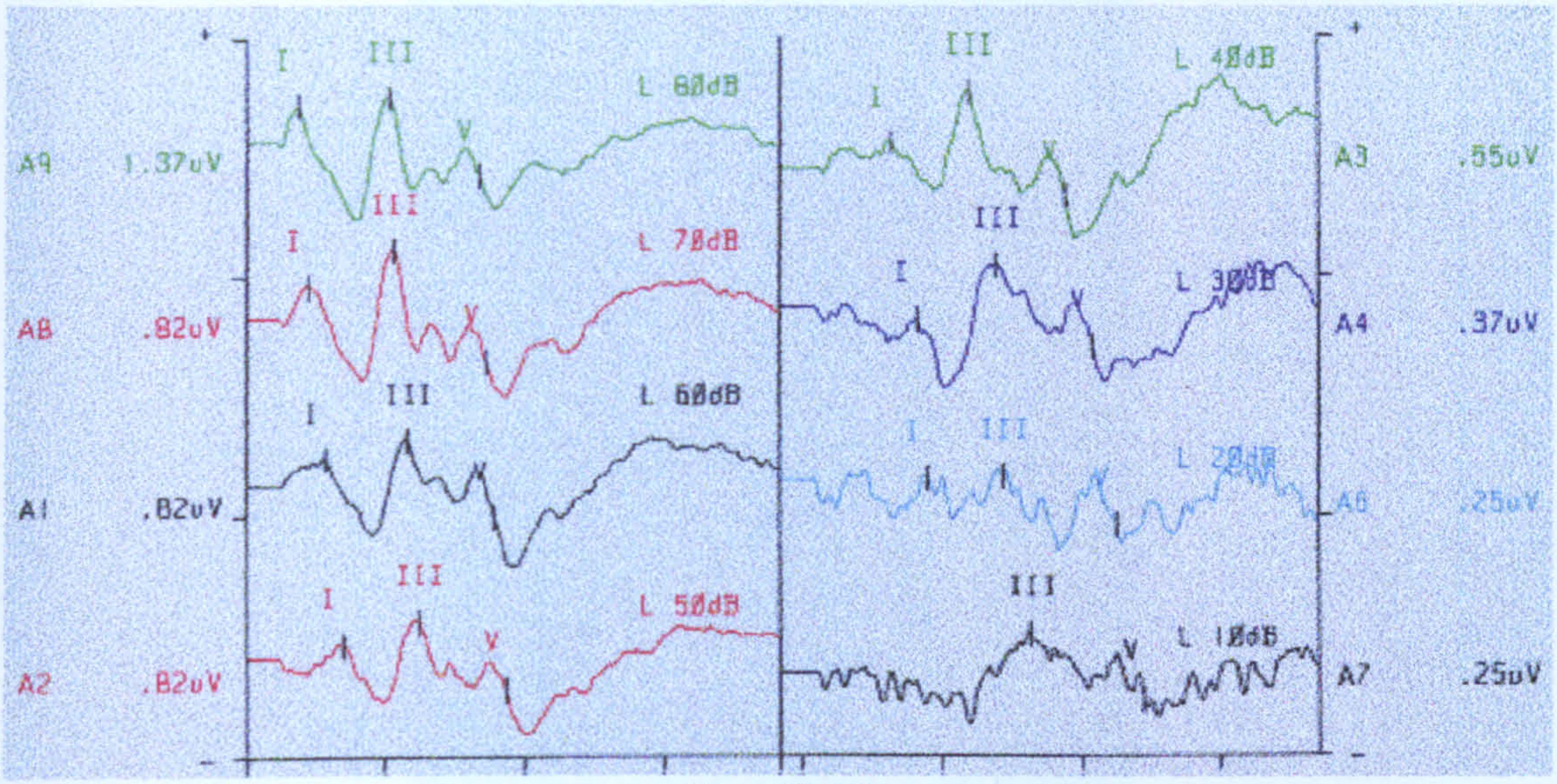
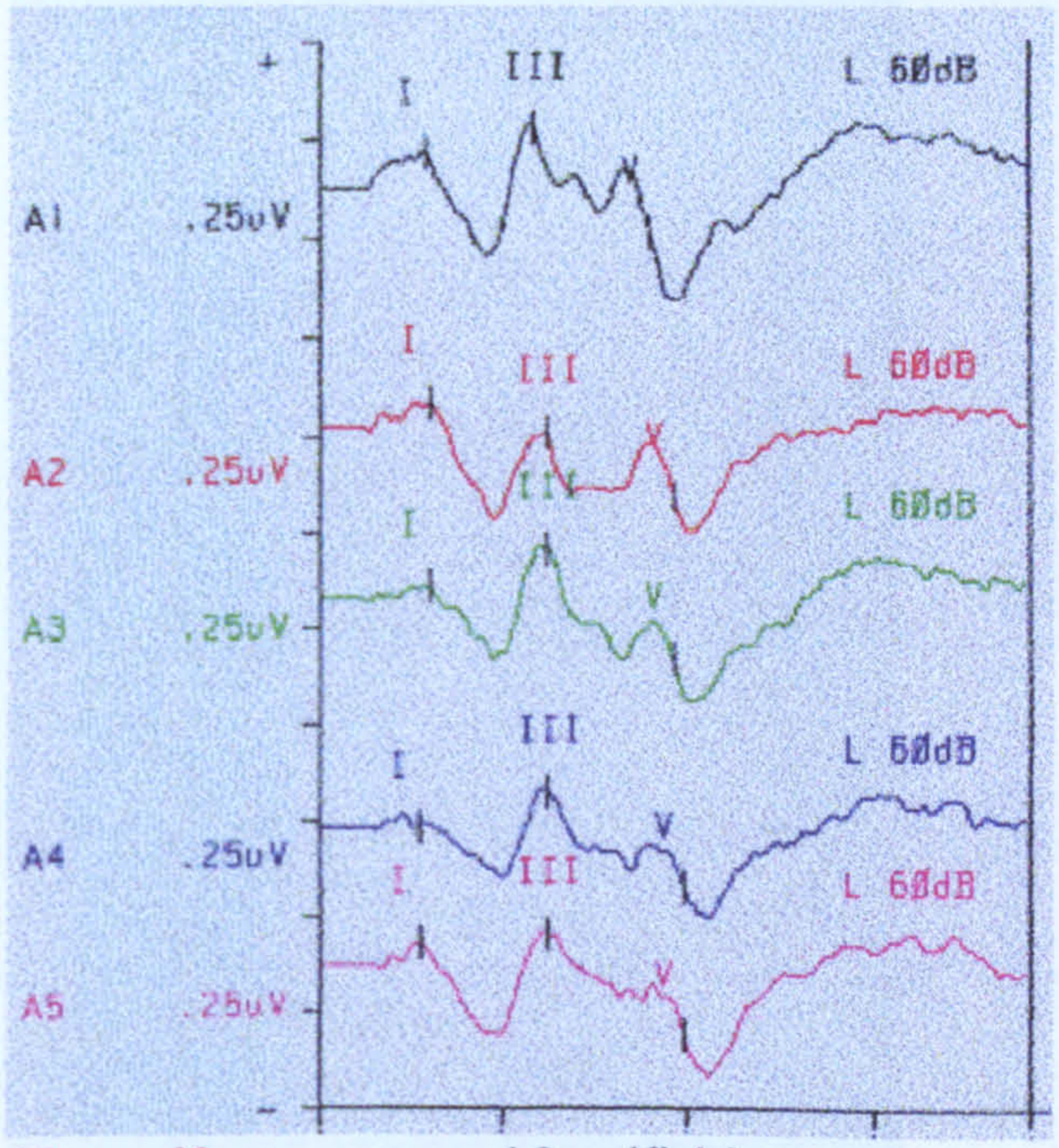
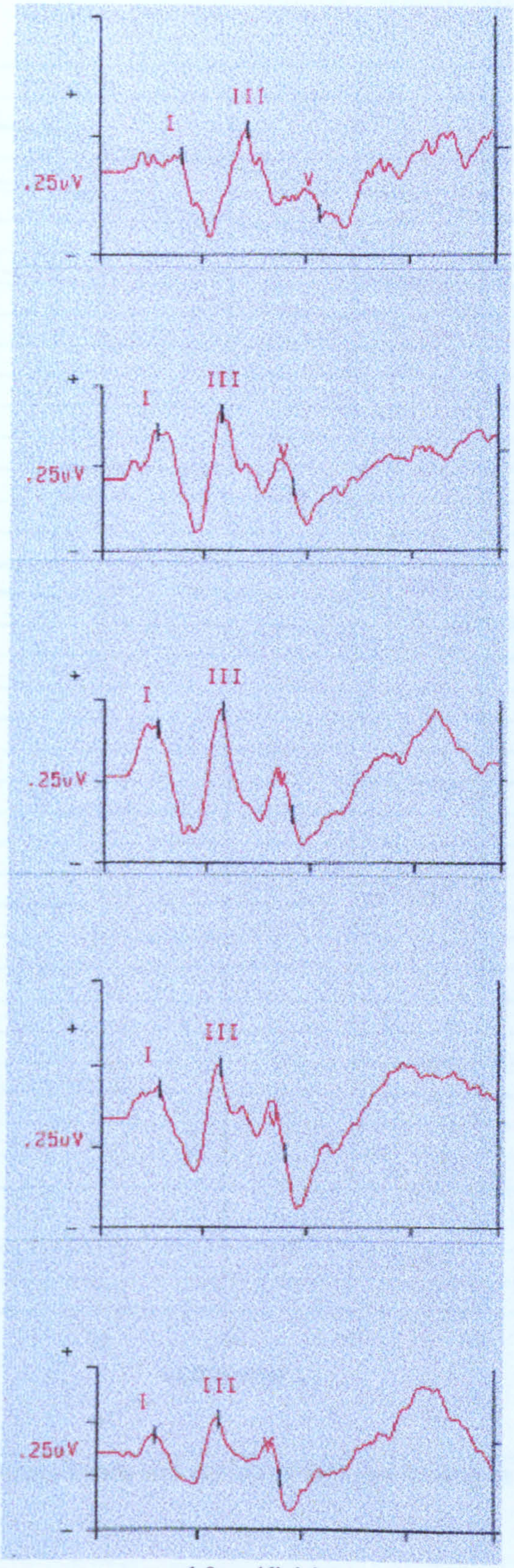


Figure 5.6 ABR for varying stimulus rates (13, 37 and 61pps) (Waves I, III and V are marked) The data shows the prolongation of latency values for increasing stimulus rate (13, 37 and 61pps). The greatest prolongation is between 13 and 37pps.



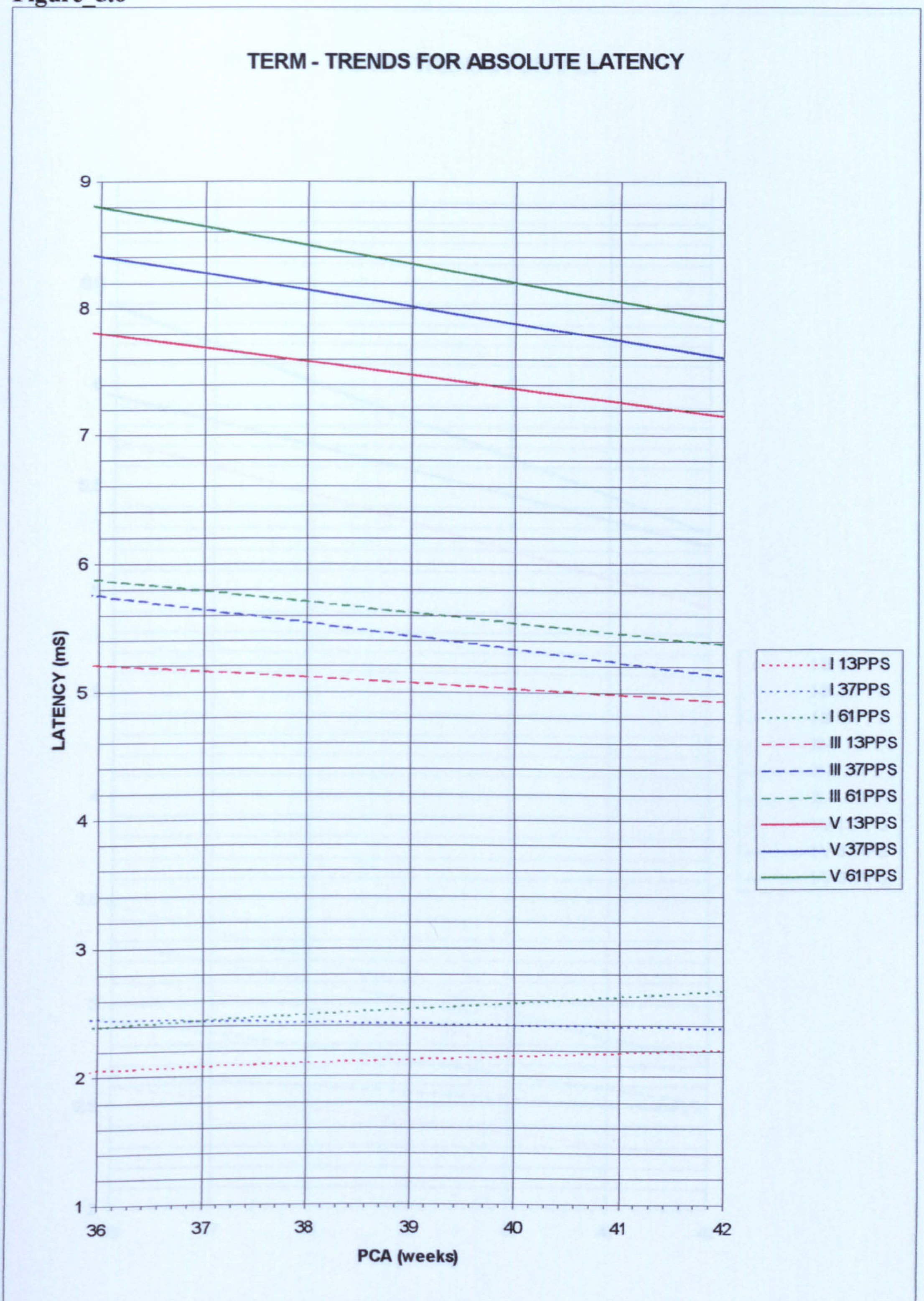
A1 13pps
A2/A3 37pps
A4/A5 61pps

Figure 5.7 ABR data for preterm infant (subject DEN, GA 30^{+2} weeks) Data (in descending order) is for PCAs of 32^{+0}, 33^{+6}, 35^{+6}, 38^{+0} and 40^{+0} weeks. The greatest reduction in wave V latency is observed between 32^{+0} and 33^{+6} weeks PCA.



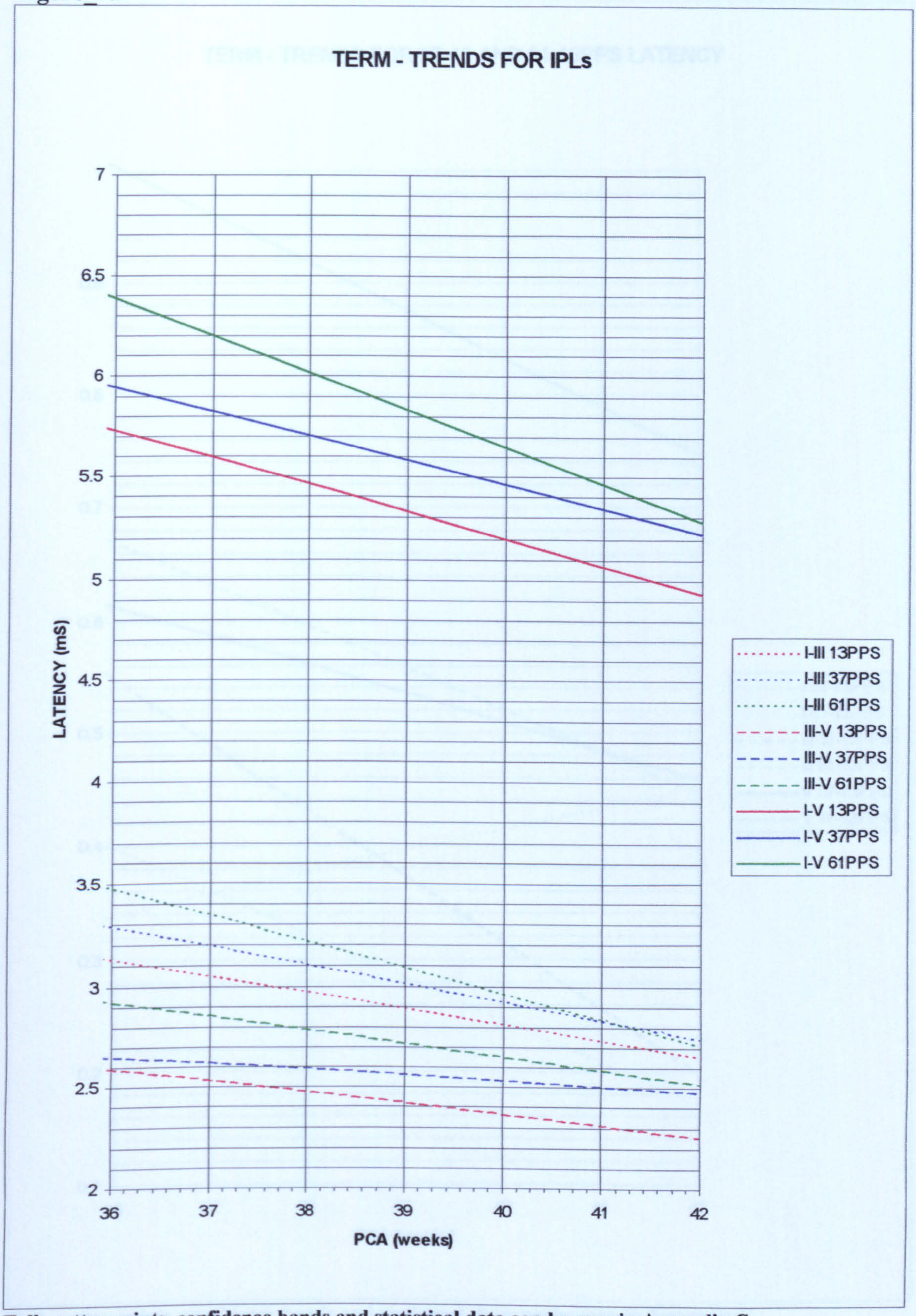
4.0 ms/division

Figure_5.8



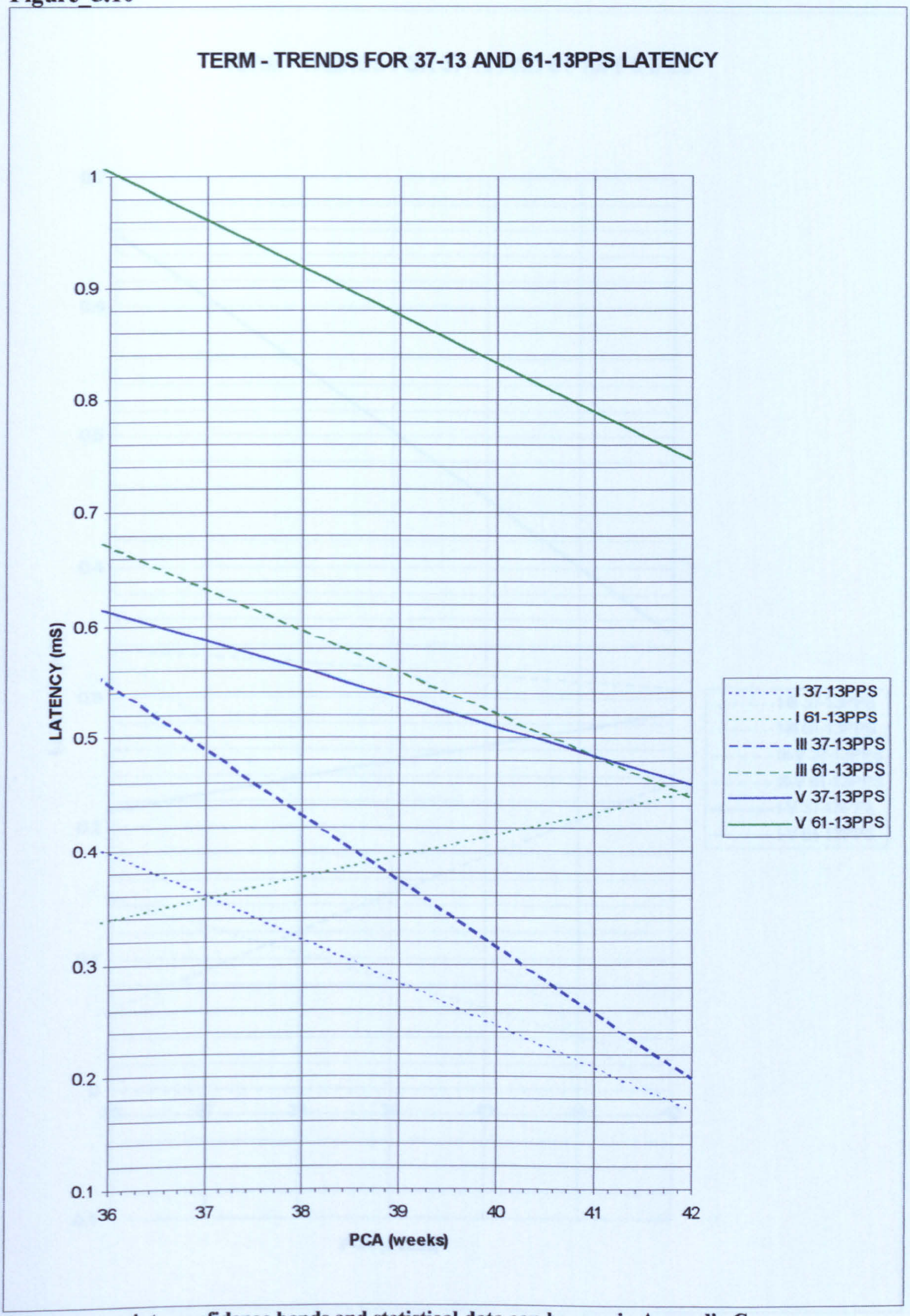
Full scatter points, confidence bands and statistical data can be seen in Appendix C.

Figure_5.9



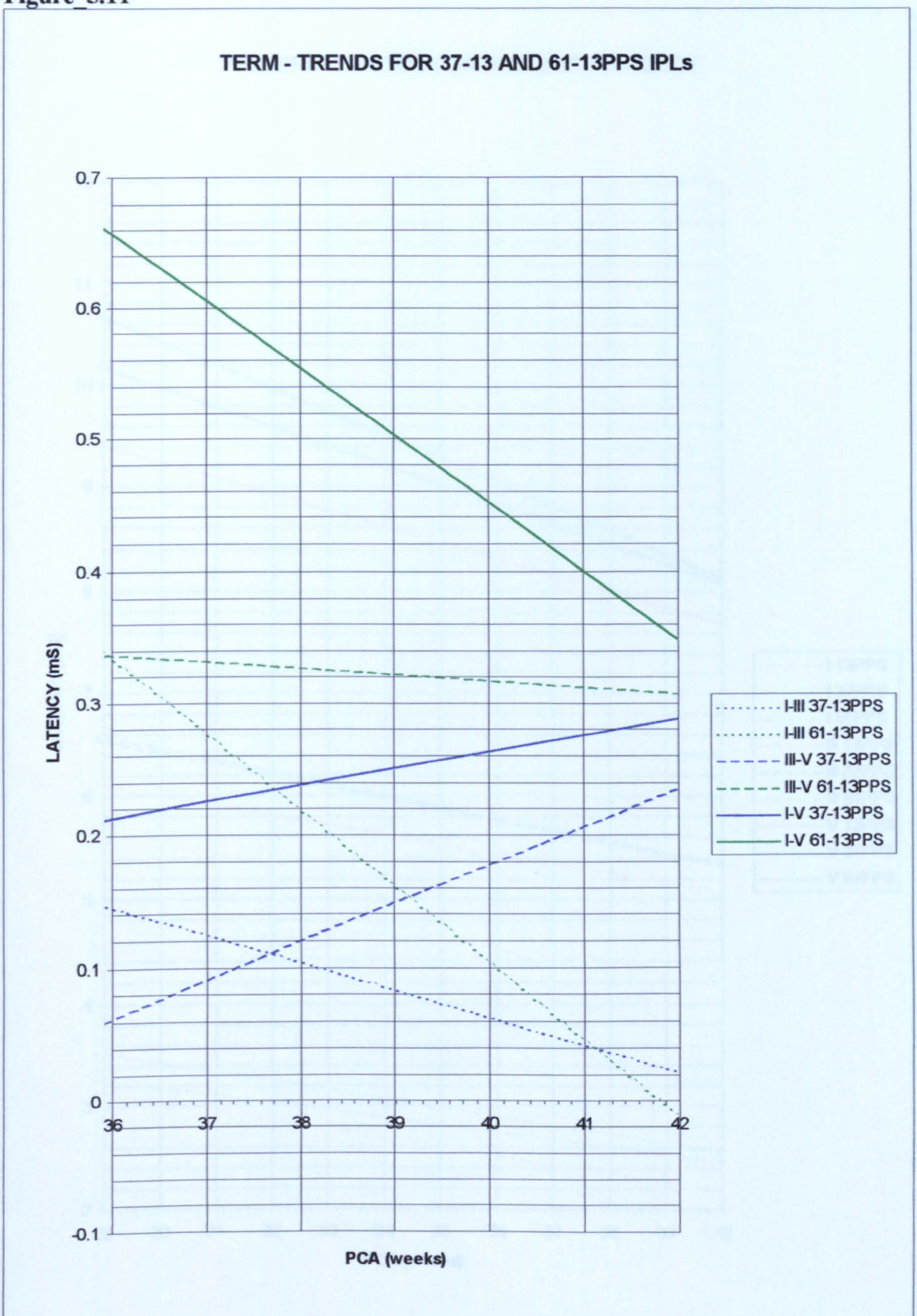
Full scatter points, confidence bands and statistical data can be seen in Appendix C.

Figure_5.10



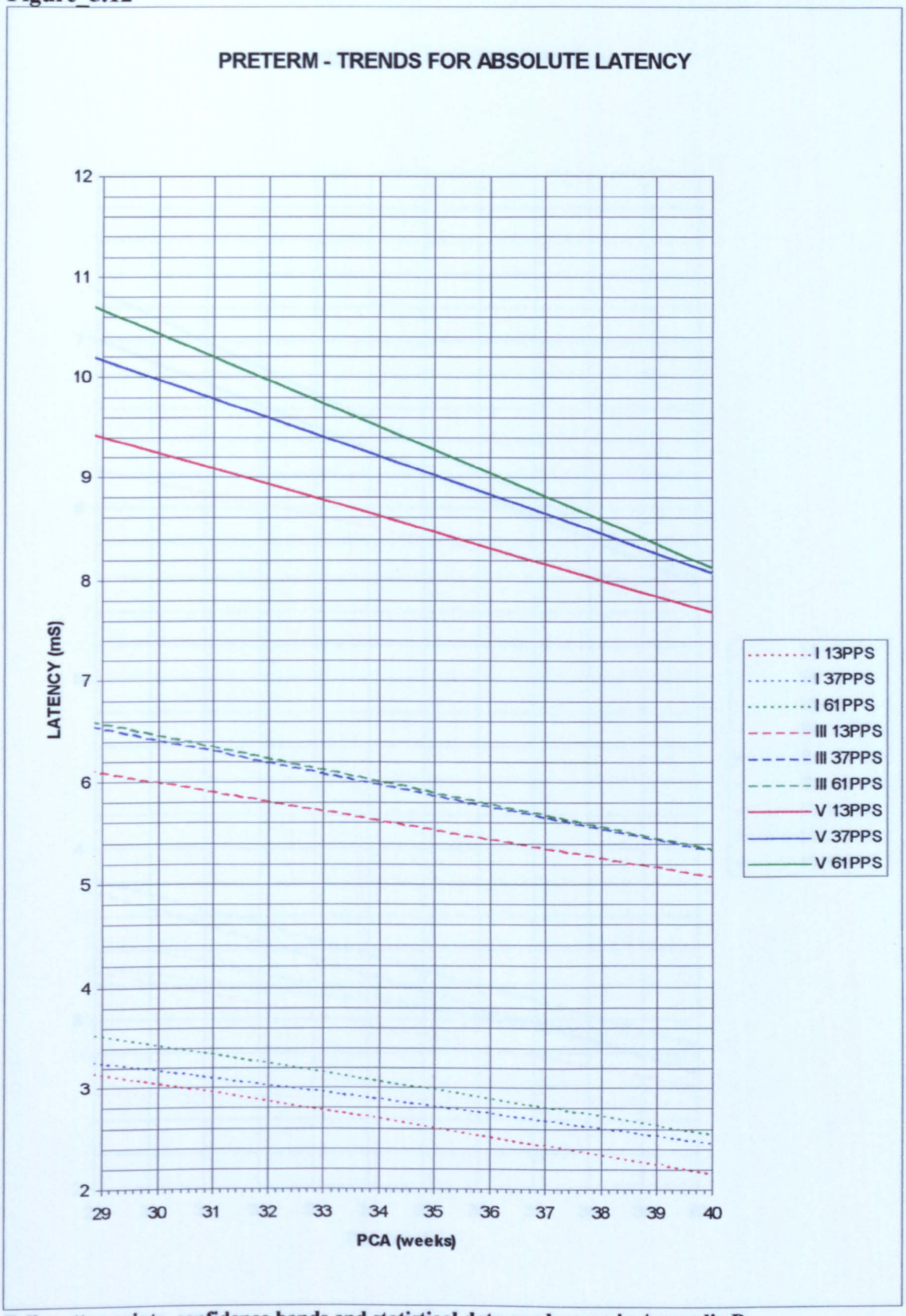
Full scatter points, confidence bands and statistical data can be seen in Appendix C.

Figure_5.11



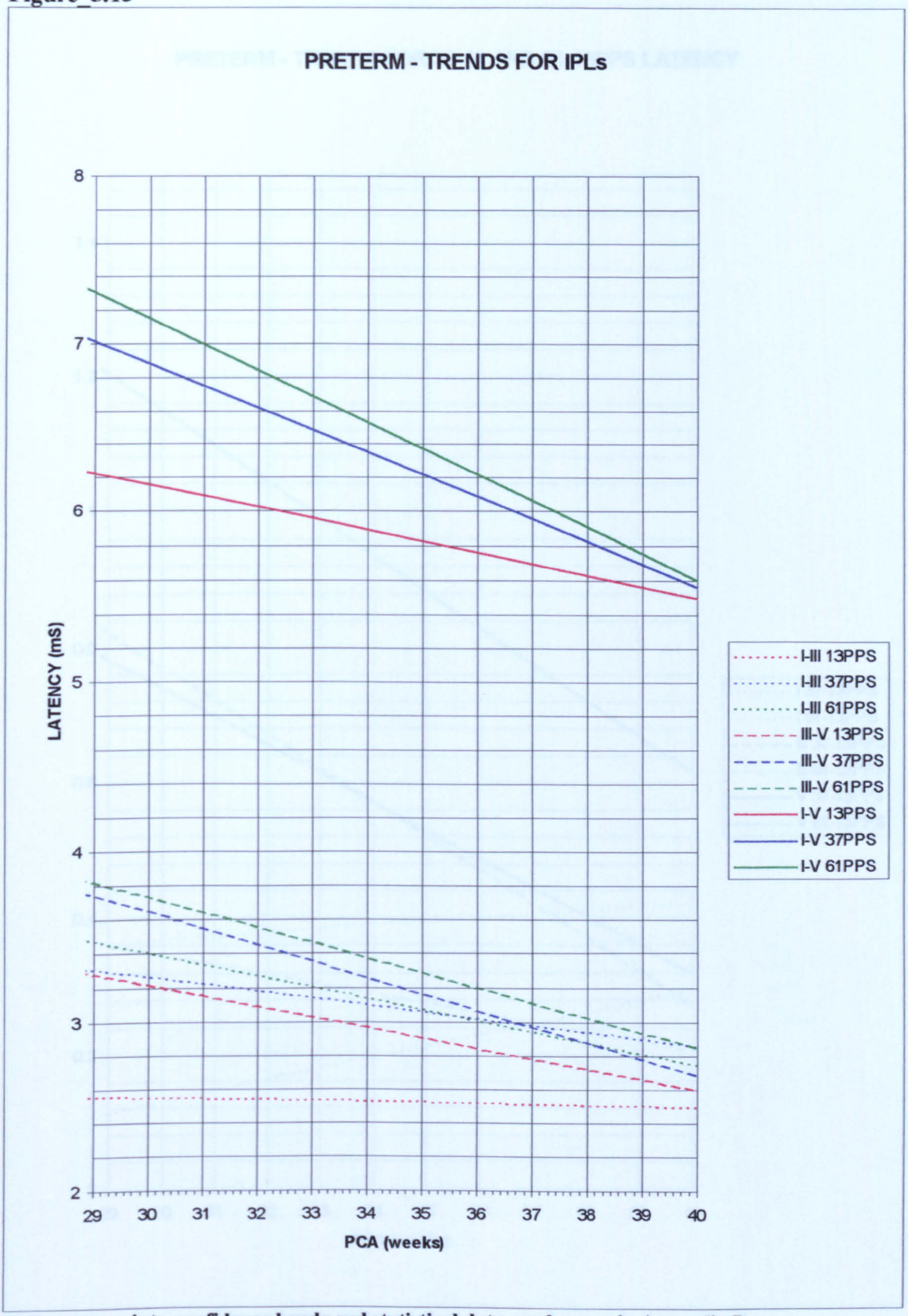
Full scatter points, confidence bands and statistical data can be seen in Appendix C.

Figure_5.12



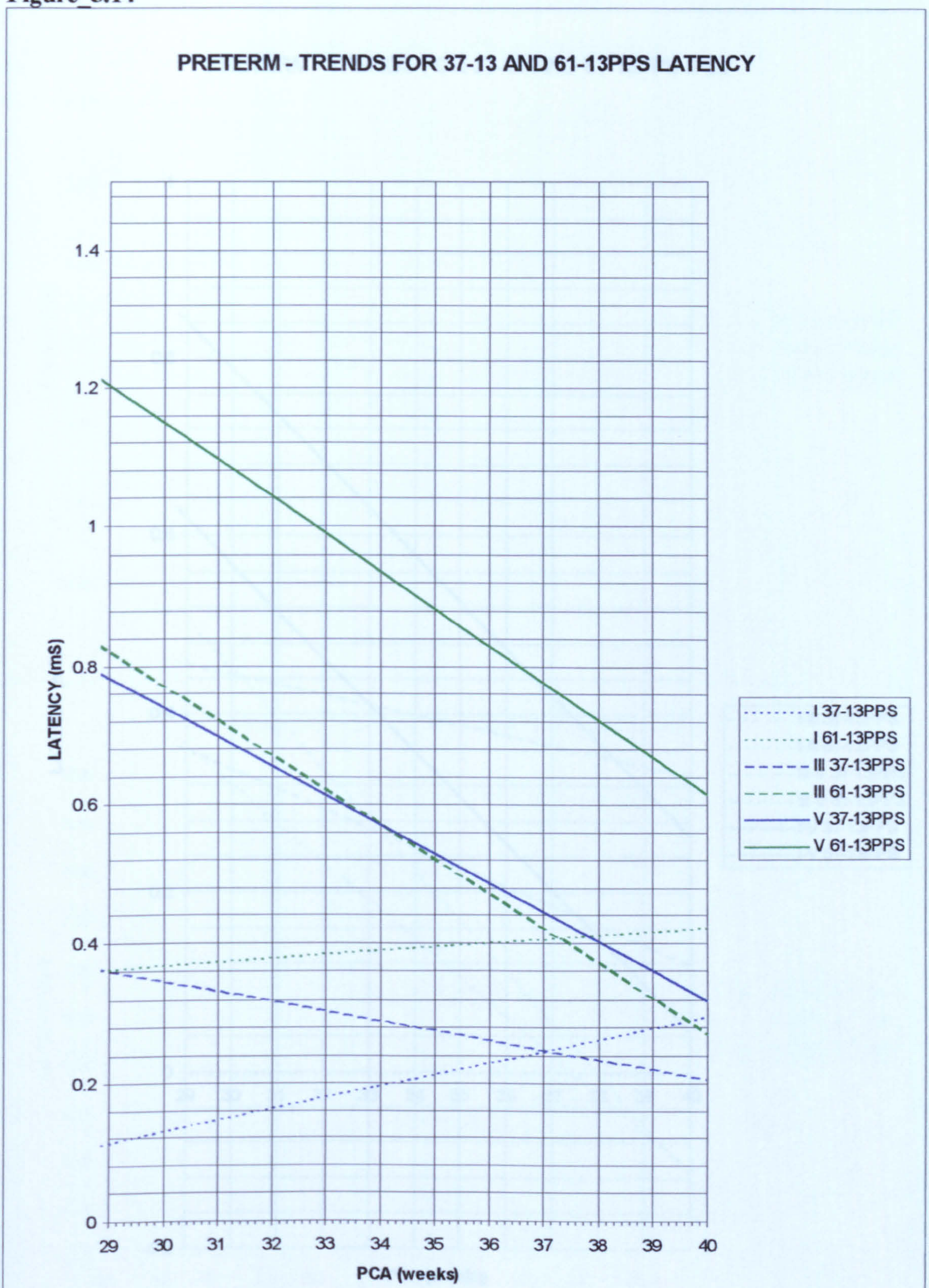
Full scatter points, confidence bands and statistical data can be seen in Appendix D.

Figure_5.13



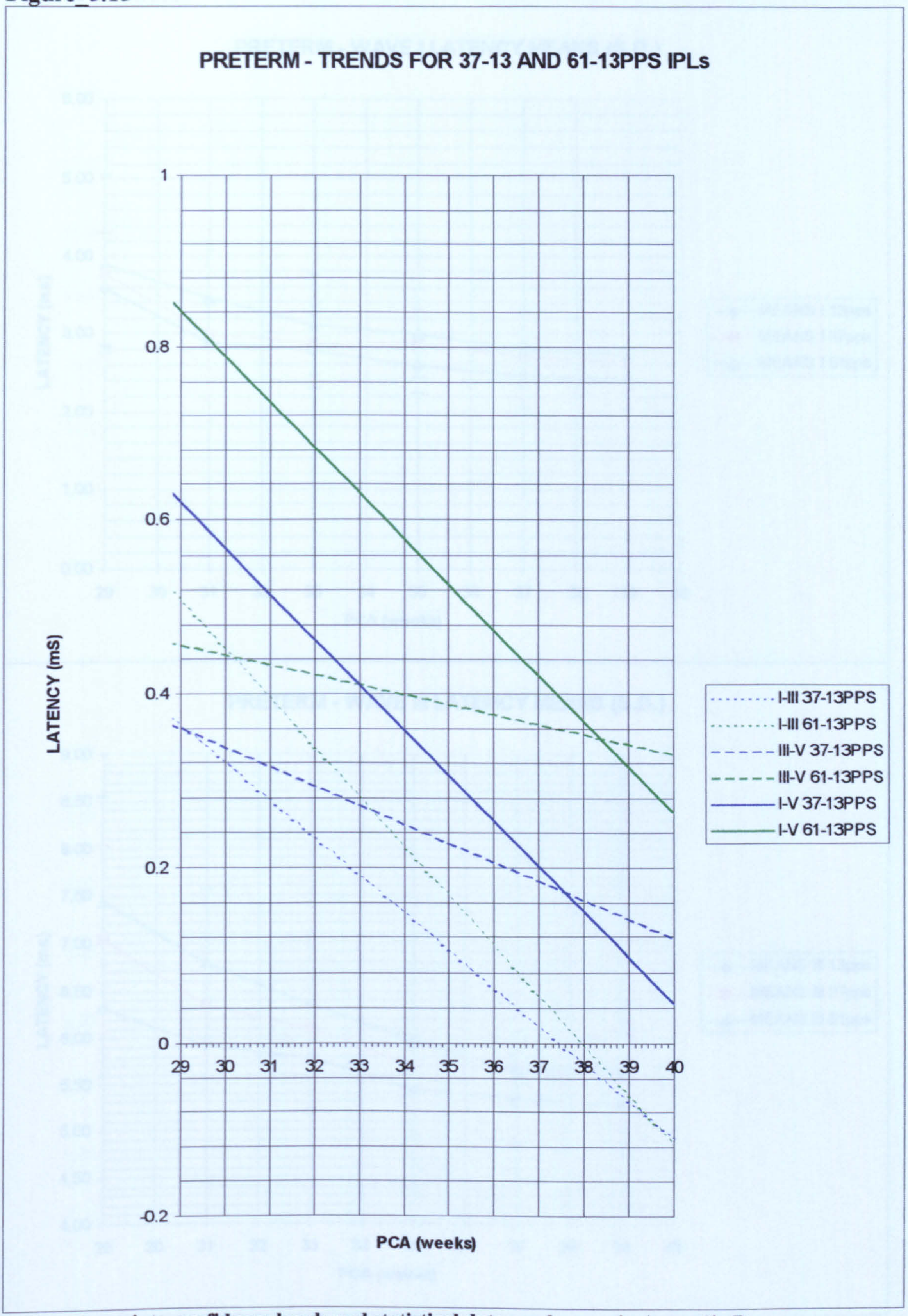
Full scatter points, confidence bands and statistical data can be seen in Appendix D.

Figure_5.14



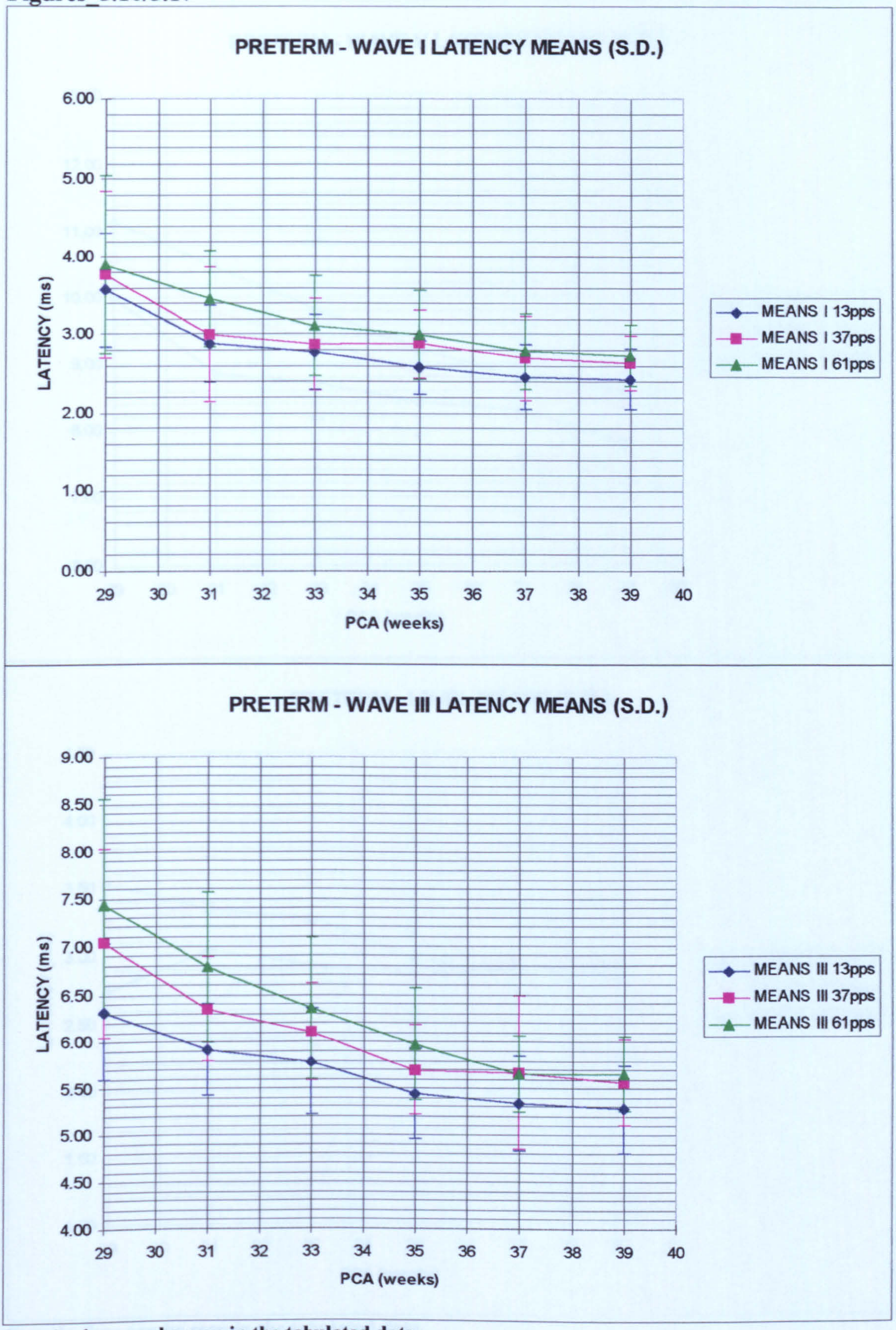
Full scatter points, confidence bands and statistical data can be seen in Appendix D.

Figure_5.15



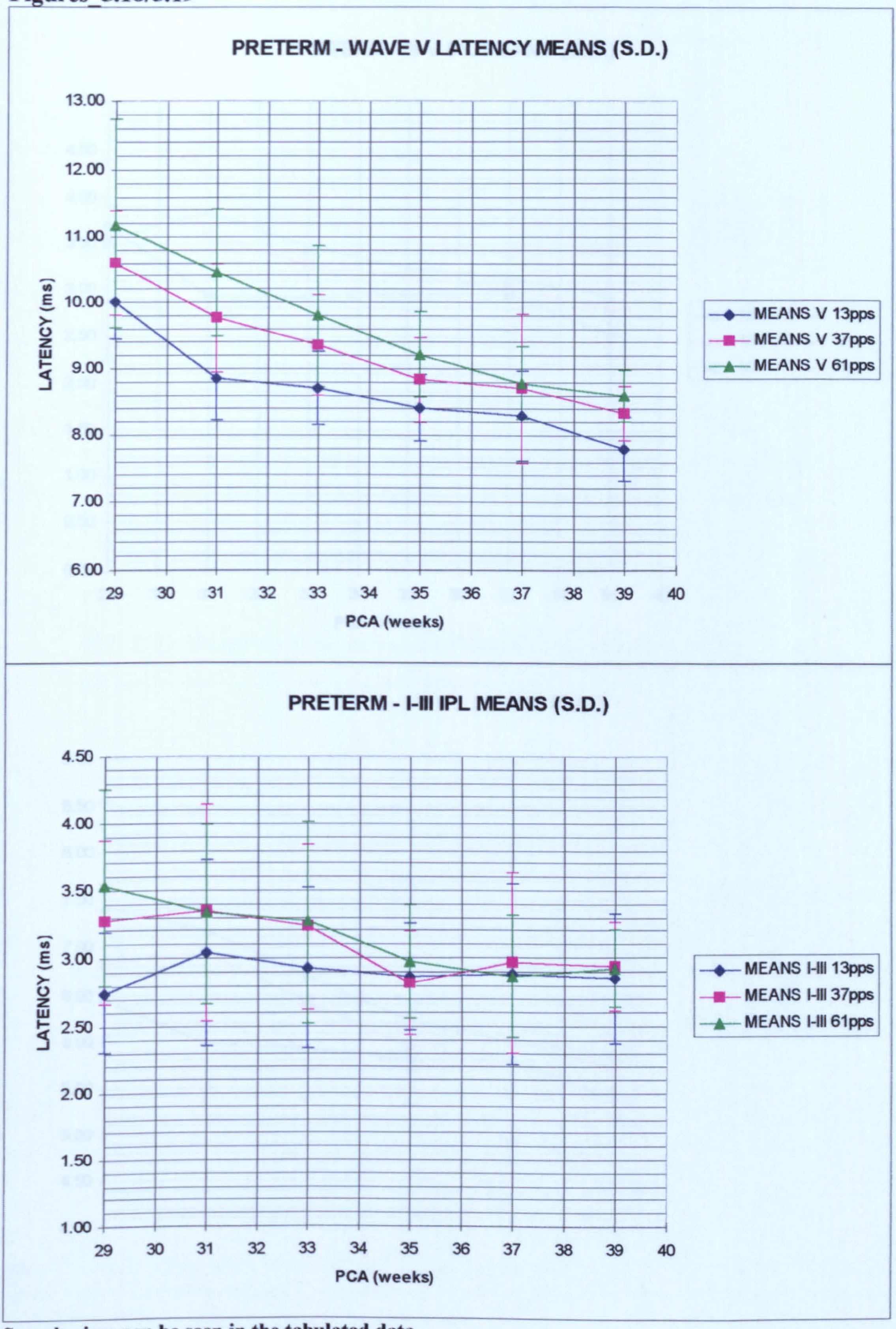
Full scatter points, confidence bands and statistical data can be seen in Appendix D.

Figures_5.16/5.17



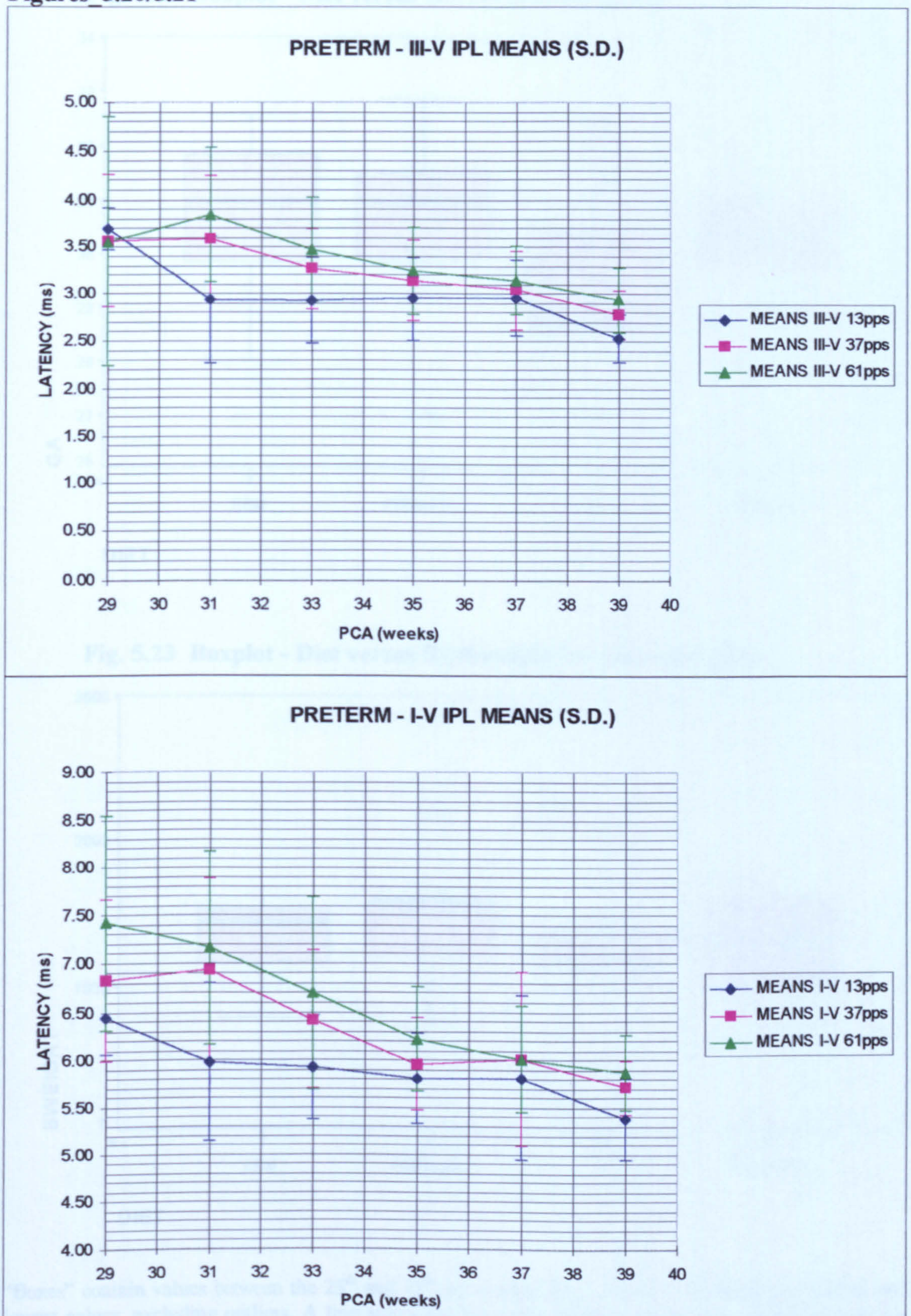
Sample sizes can be seen in the tabulated data.

Figures_5.18/5.19



Sample sizes can be seen in the tabulated data.

Figures_5.20/5.21



Sample sizes can be seen in the tabulated data.

Fig. 5.22 Boxplot - Diet versus GA for preterm group

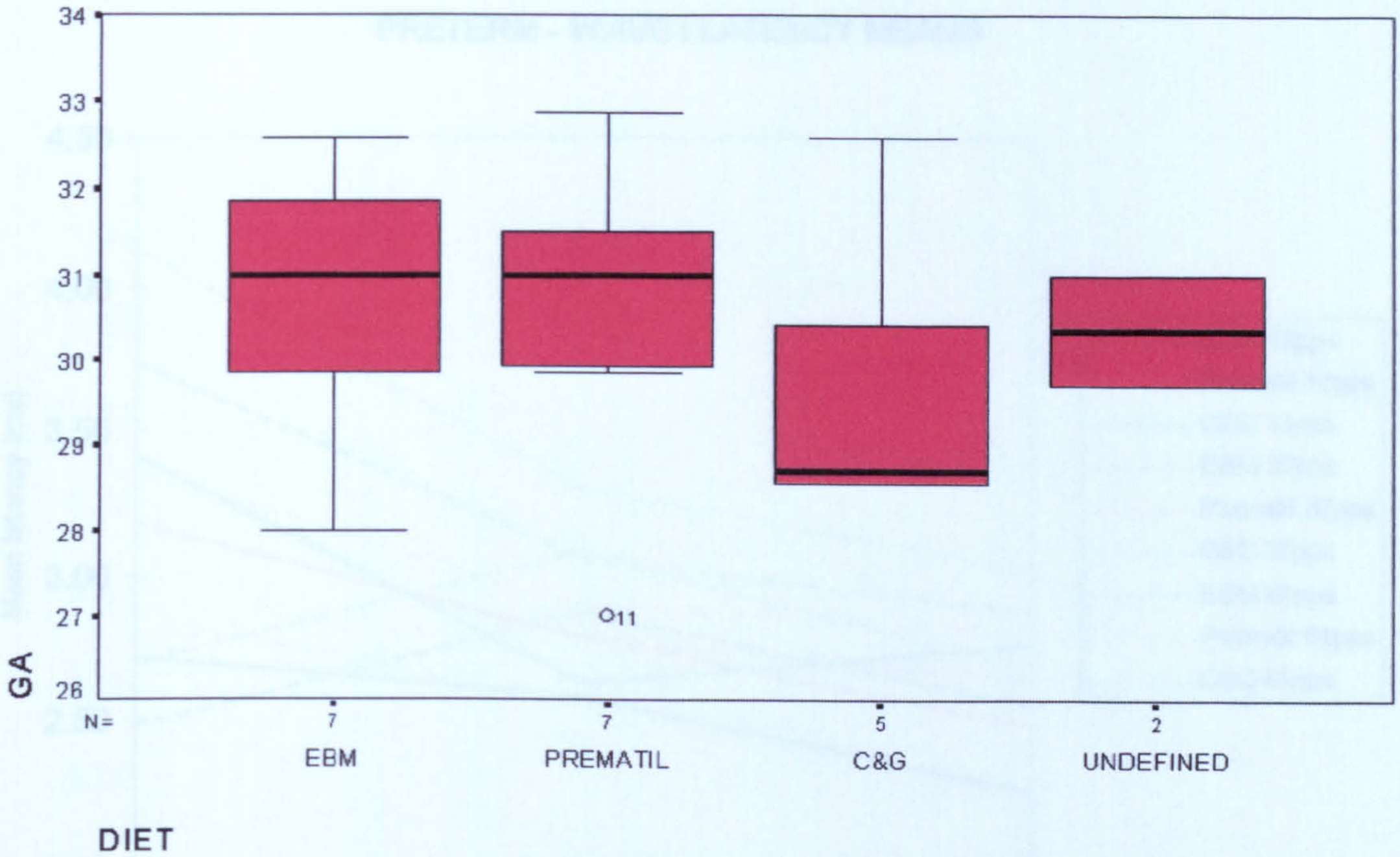
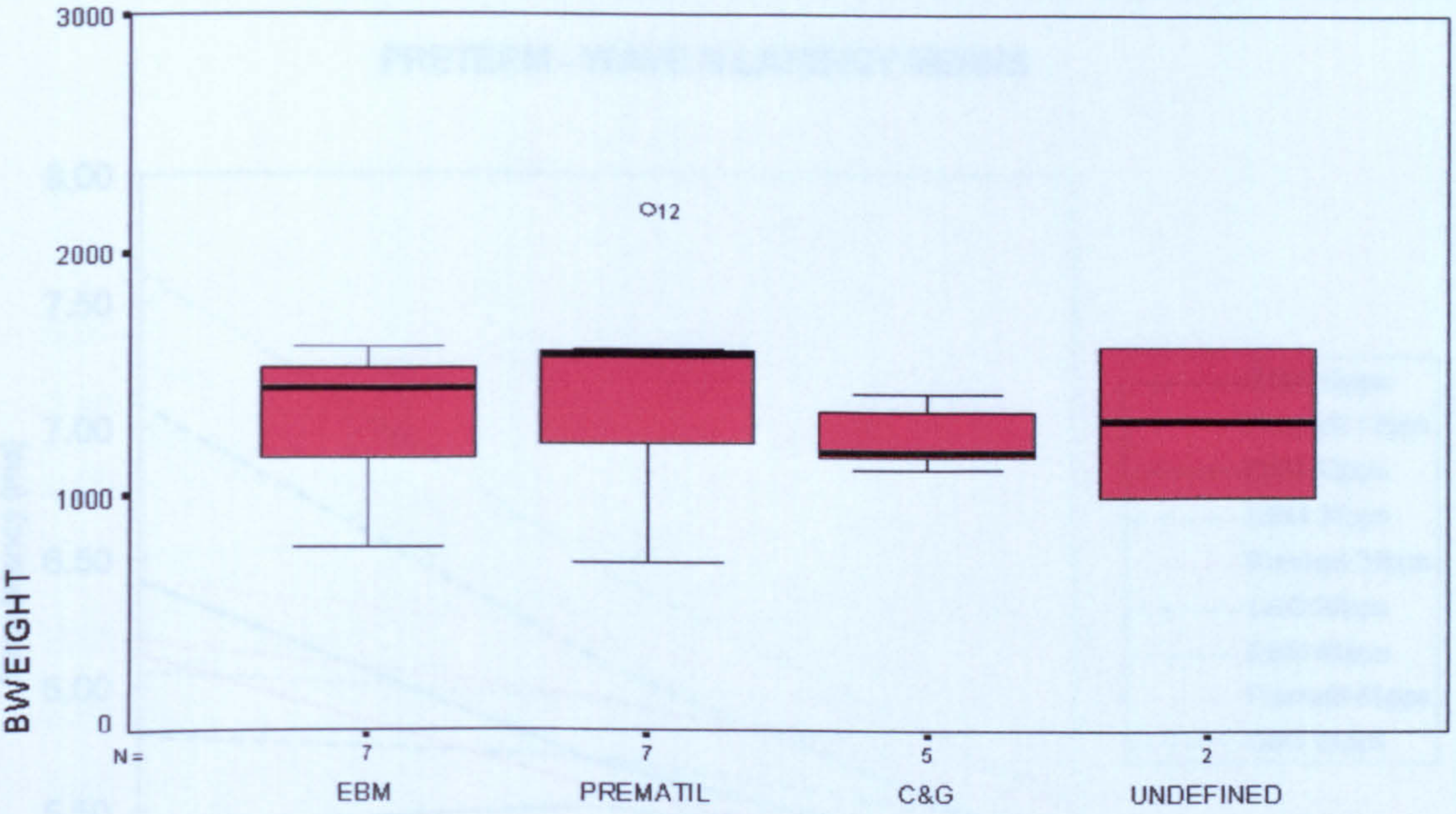
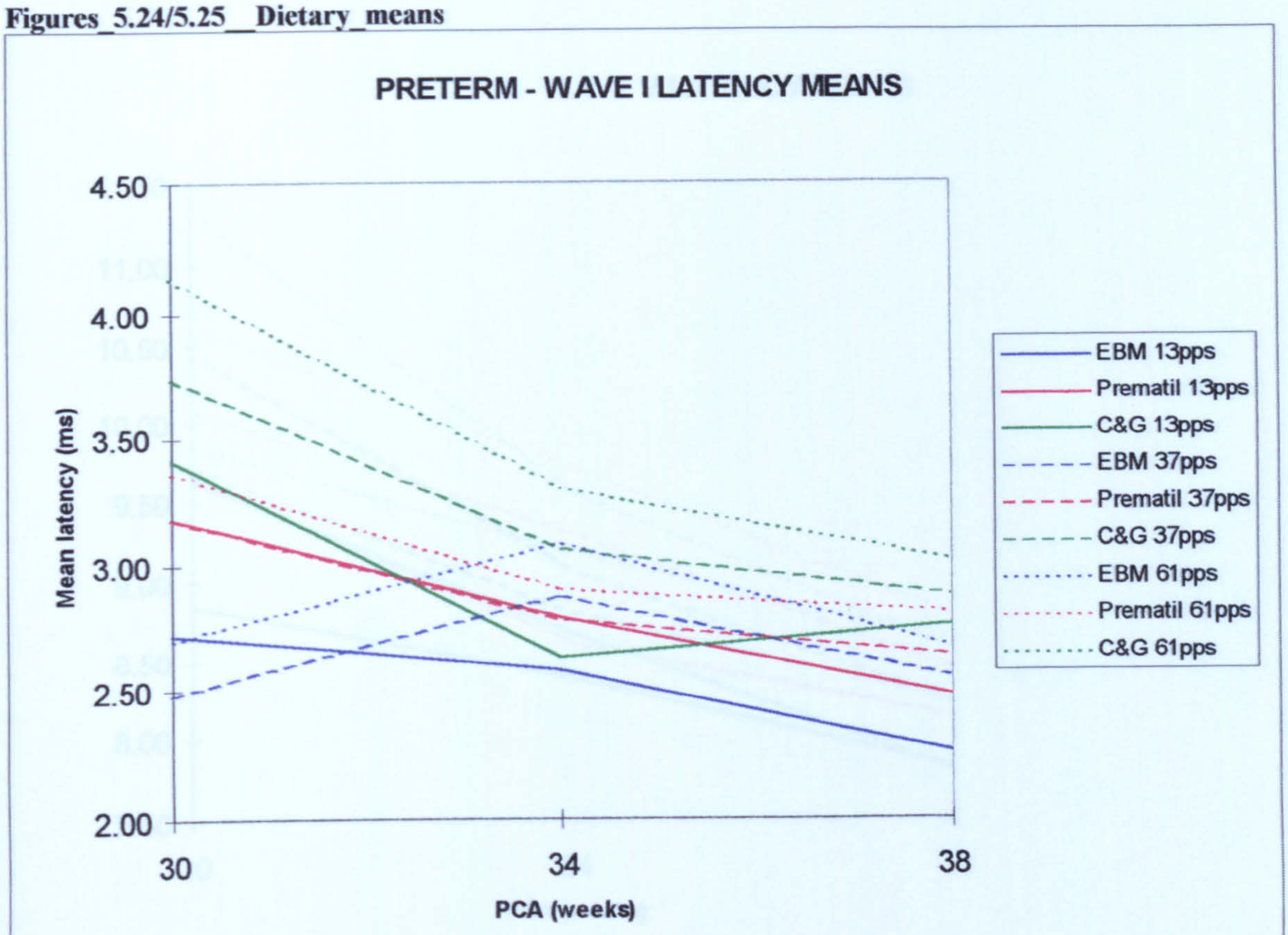


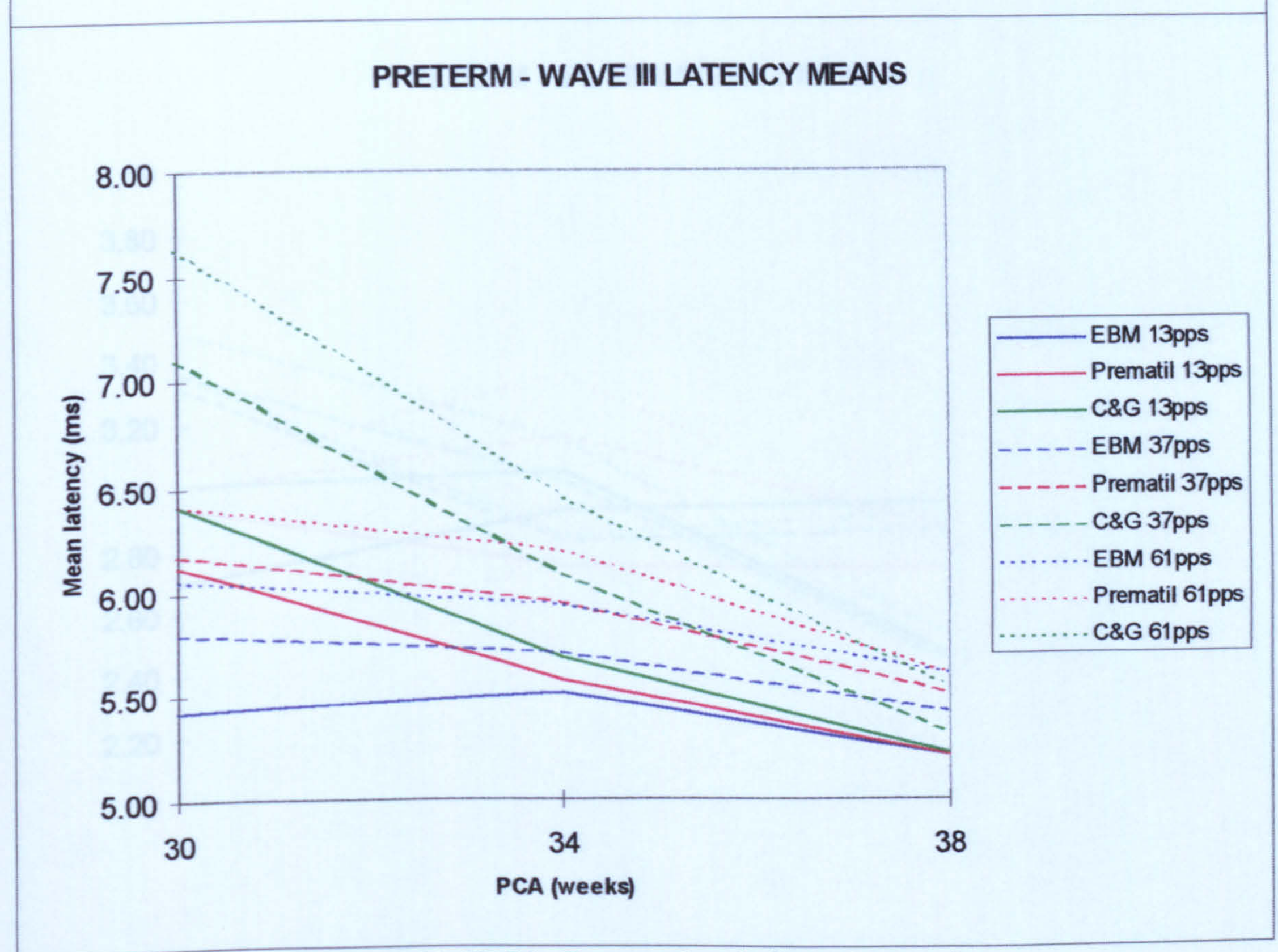
Fig. 5.23 Boxplot - Diet versus Birthweight for preterm group



"Boxes" contain values between the 25th and 75th percentiles, the "whiskers" extend to the highest and lowest values, excluding outliers. A line across the box indicates the median. Any outliers present are plotted separately.

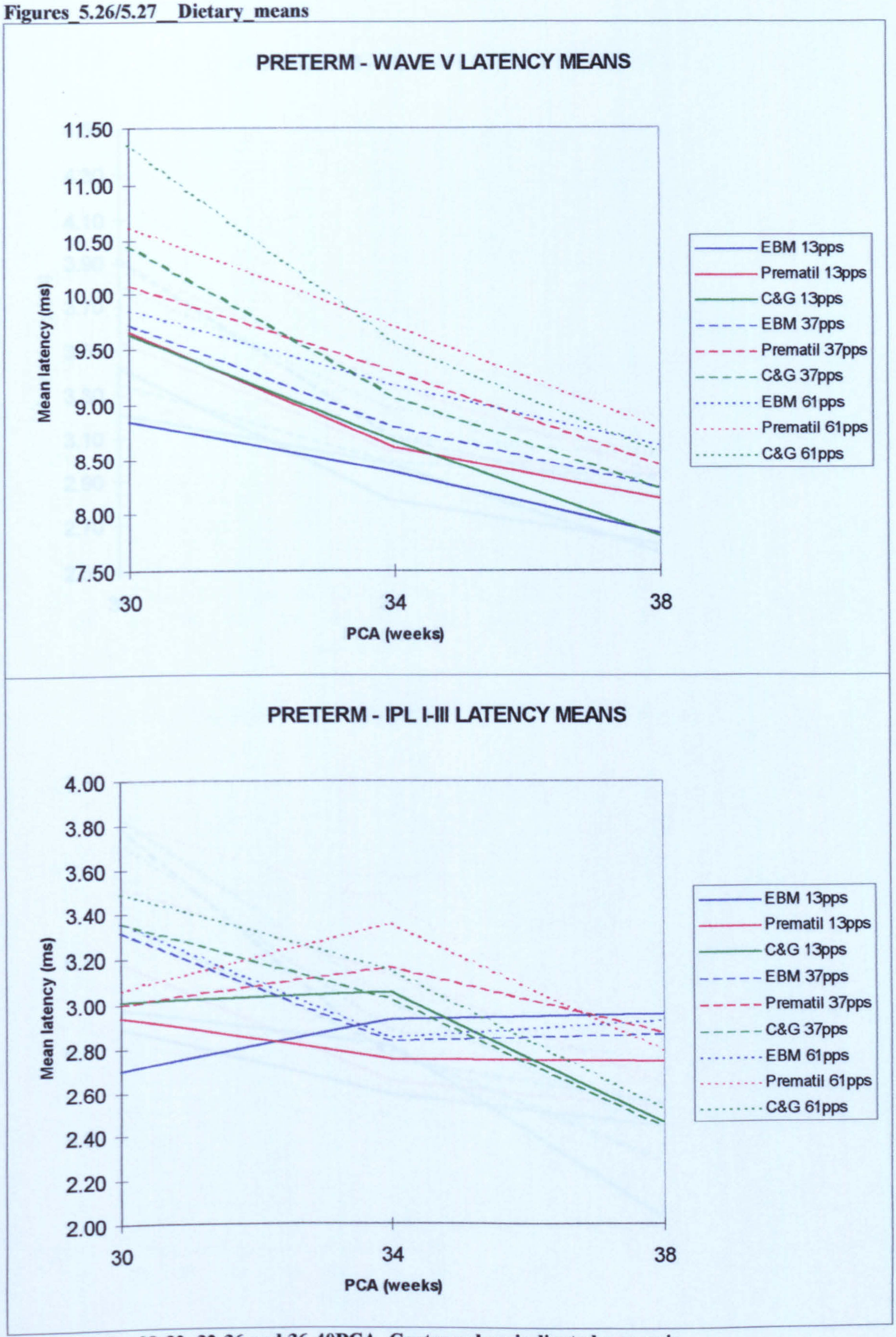
Figures_5.24/5.25__Dietary_means





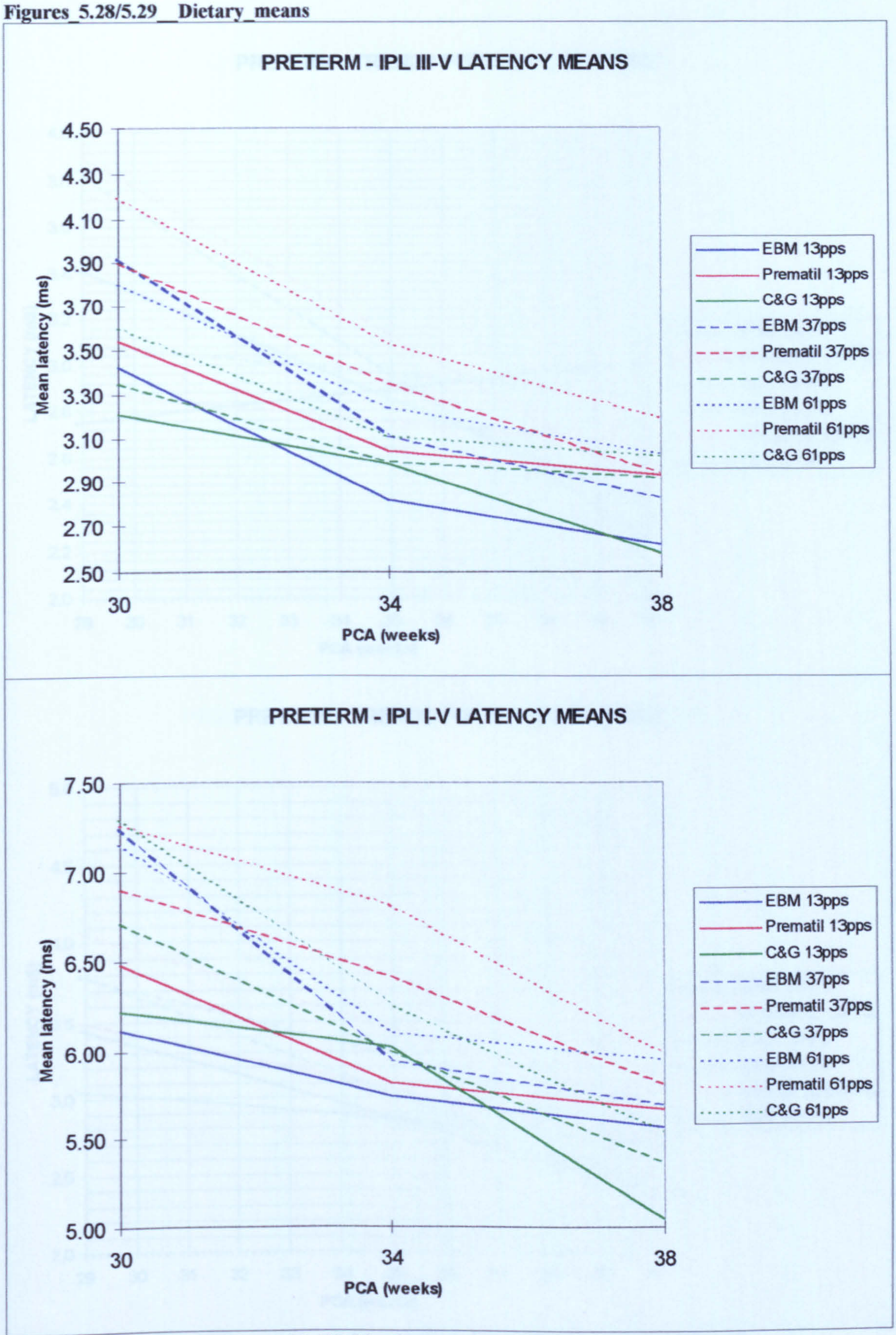
Means are for 28-32, 32-36 and 36-40PCA. Centre values indicated on x-axis.
Sample sizes can be seen in the tabulated data.

Figures_5.26/5.27__Dietary_means



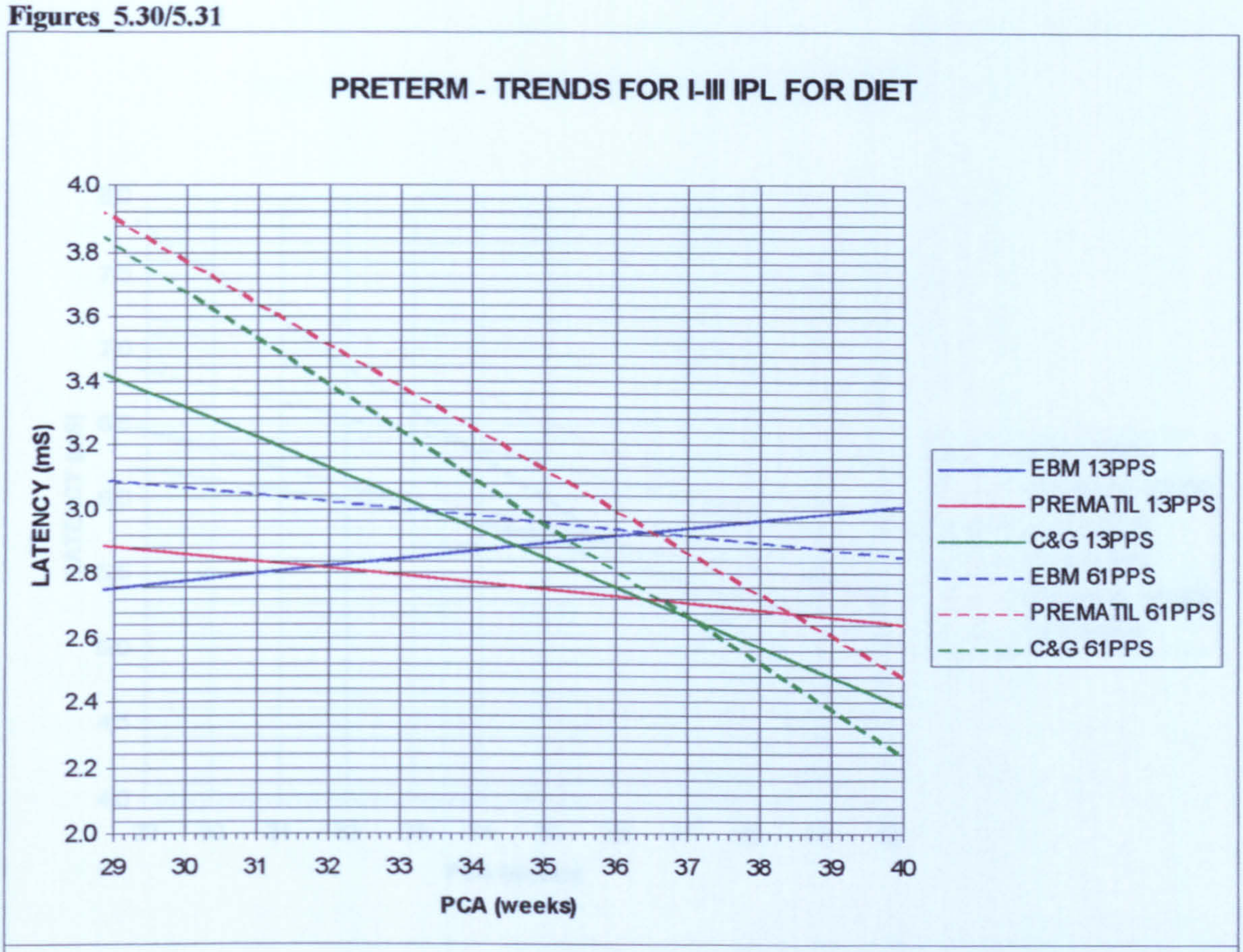
Means are for 28-32, 32-36 and 36-40PCA. Centre values indicated on x-axis.
Sample sizes can be seen in the tabulated data.

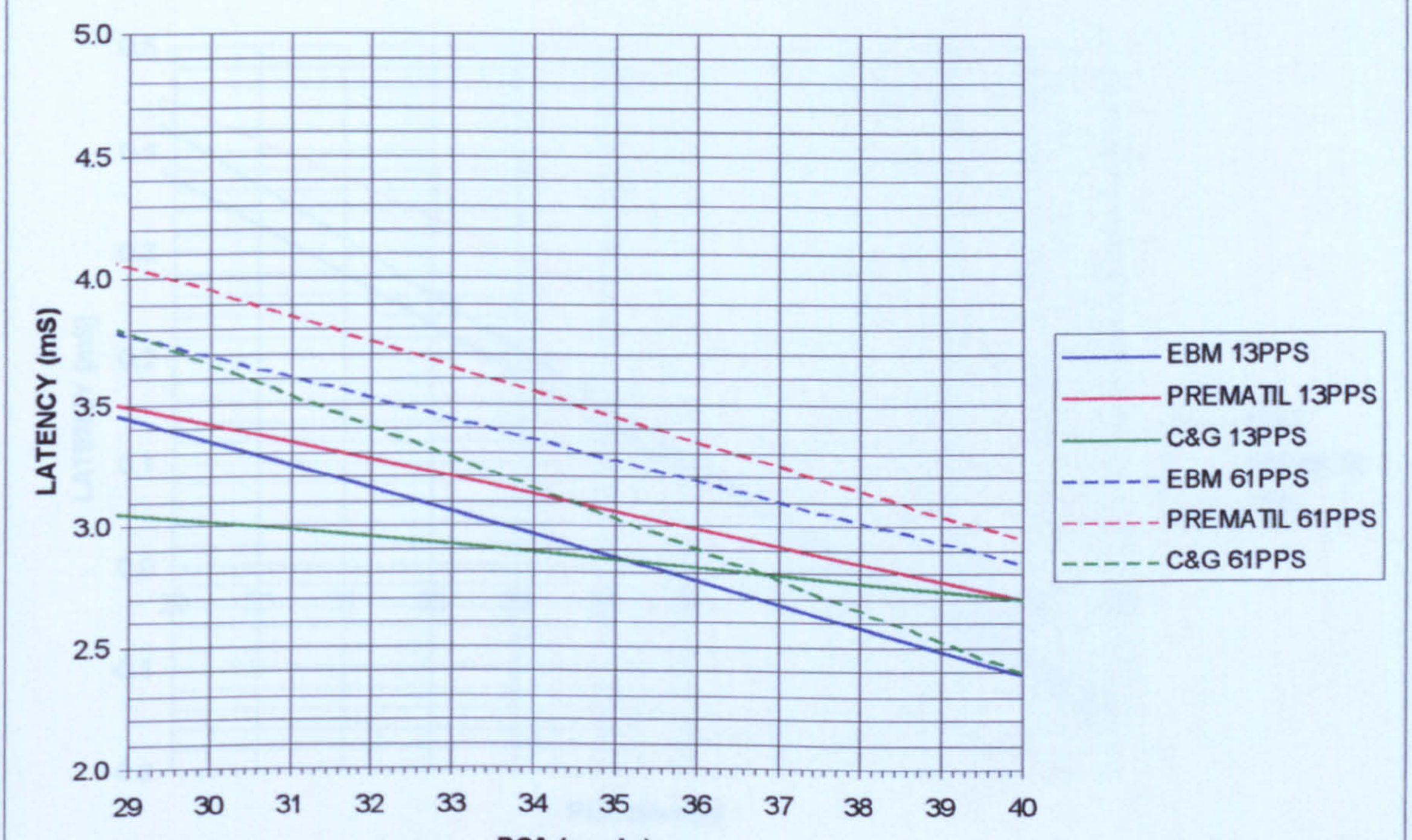
Figures_5.28/5.29__Dietary_means



Means are for 28-32, 32-36 and 36-40PCA. Centre values indicated on x-axis.
Sample sizes can be seen in the tabulated data.

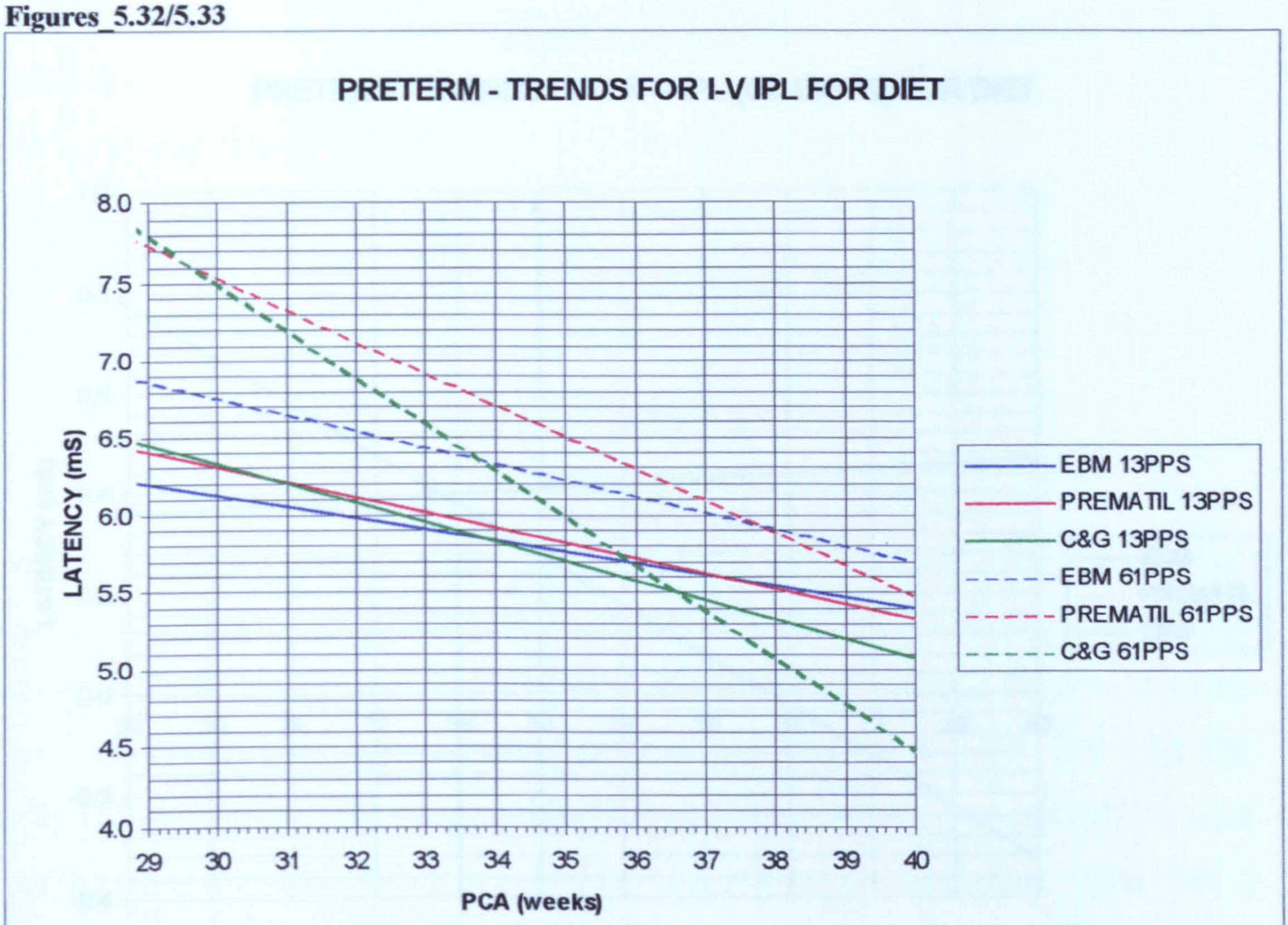
Figures_5.30/5.31

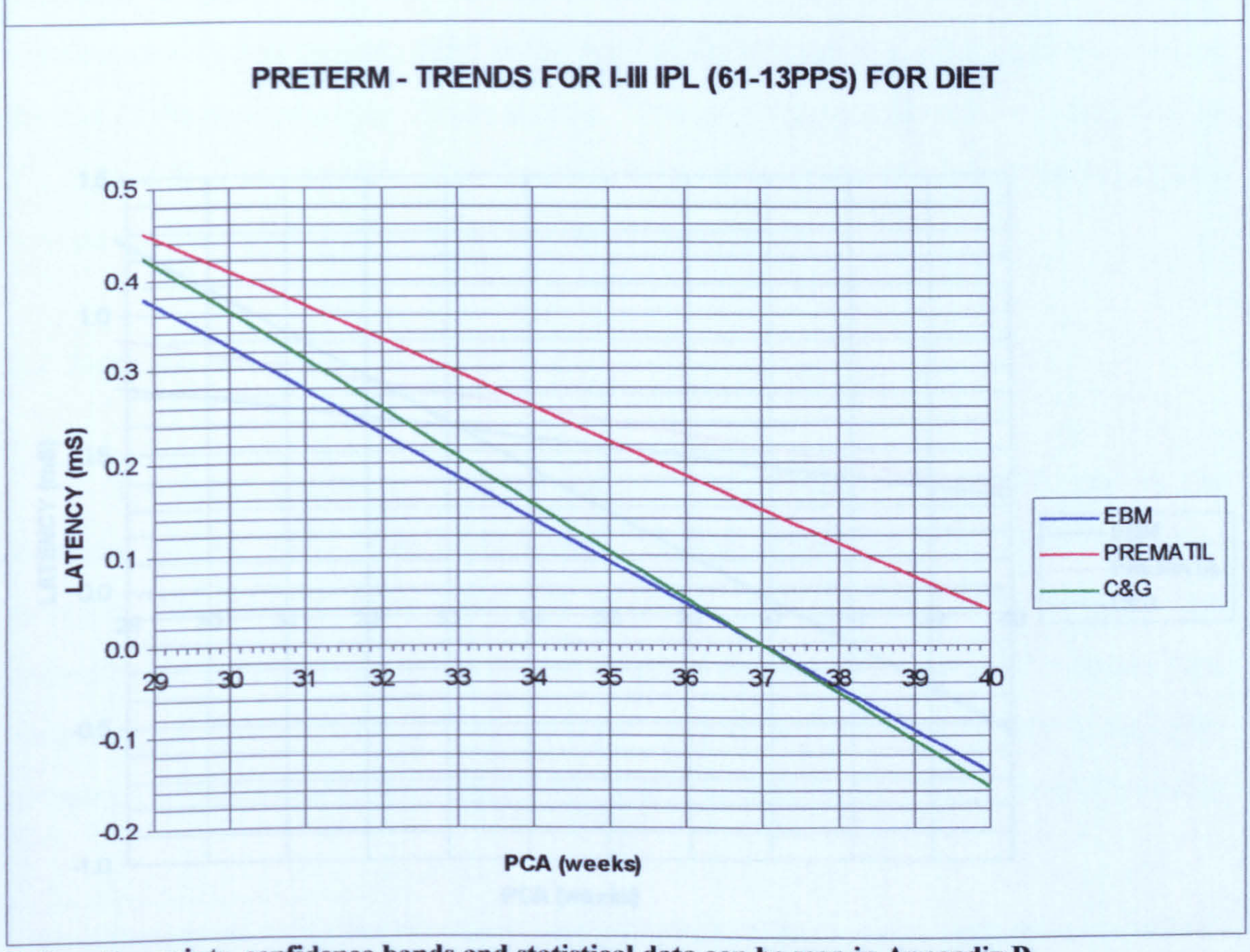




Full scatter points, confidence bands and statistical data can be seen in Appendix D.

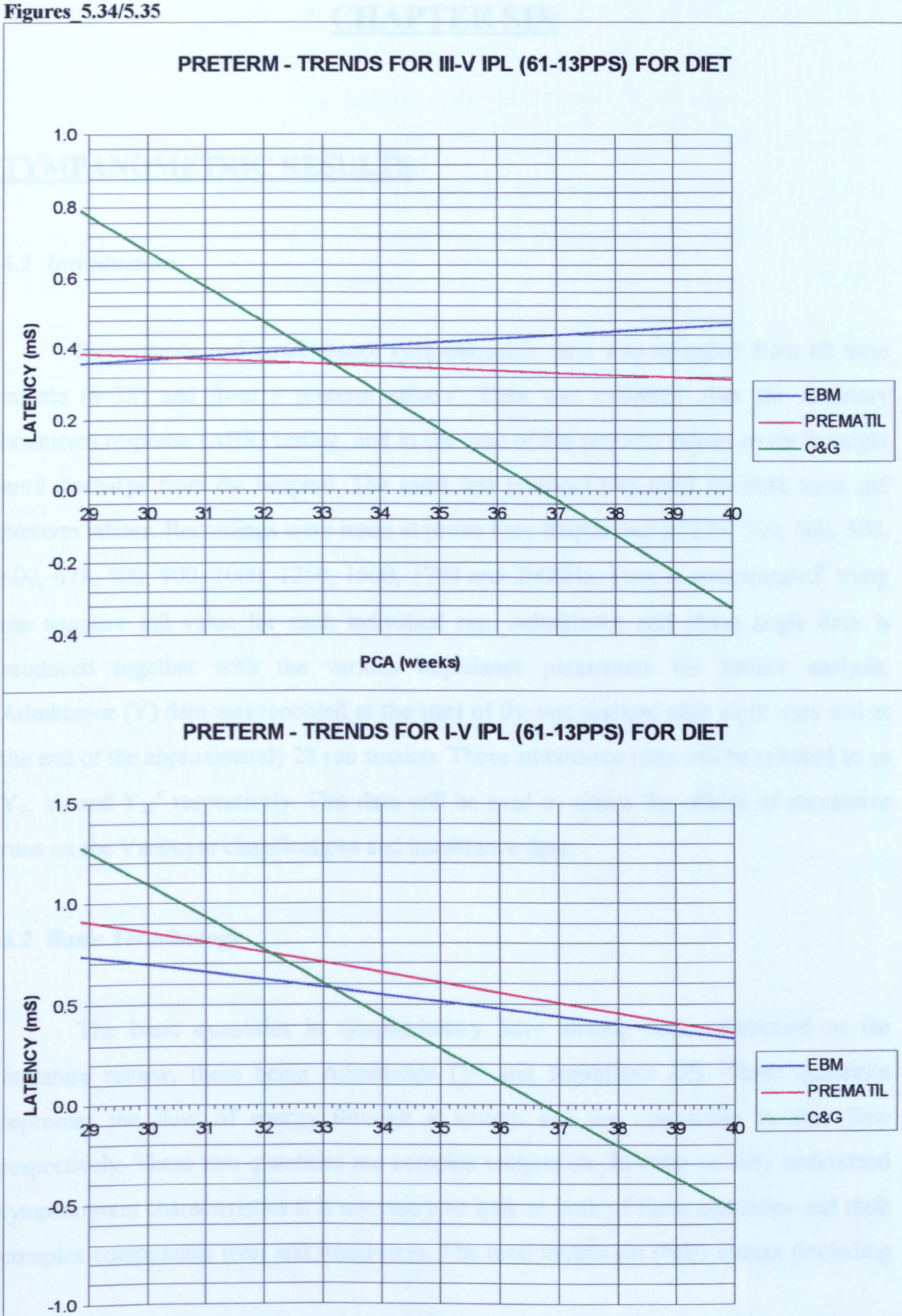
Figures_5.32/5.33





Full scatter points, confidence bands and statistical data can be seen in Appendix D.

Figures_5.34/5.35



Full scatter points, confidence bands and statistical data can be seen in Appendix D.

CHAPTER SIX

TYMPANOMETRIC RESULTS

6.1 Introduction

Susceptance and conductance tympanometric data was recorded from all term infants (n=28) and from 8 preterm infants[a]. Data was collected after the Auditory brainstem response (ABR) testing, and in the case of the preterm infants every fortnight until discharge from the hospital. The same test protocol was used for both term and preterm infants. Recordings were made at probe tone frequencies of 226, 300, 400, 500, 600, 678, 800, 900, 1000, 1250, 1500, 1750 and 2000Hz. Data is compensated[b] using the negative tail value for each individual run. Admittance and phase angle data is produced together with the various impedance parameters for further analysis. Admittance (Y) data was recorded at the start of the test session, after eight tests and at the end of the approximately 28 run session. These admittance tests will be referred to as Y_1, Y_8 and Y_{28}[c] respectively. This data will be used to assess the effects of successive runs on the Vanhuyse classifications and immittance data.

6.2 Basic Terminology

The basic quantities in tympanometry have already been mentioned in the literature review, these being Admittance (Y) and Impedance (Z). These quantities represent the flow of energy through a system and the opposition to that flow respectively. These two quantities are complex reciprocals. In order to fully understand tympanogram characteristics it is necessary to look at both of these quantities and their complex components (real and imaginary). The total middle ear (ME) system (including

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[a] The tympanometric testing was introduced part way through the preterm study. All subsequent infants provided full data.

[b] Refer to Section 3.3.3 and Appendix B for theory behind the compensation of tympanometric data.

[c] Y_{28} could actually be greater than 28 runs due to repeated runs performed with non-cooperative subjects. A number of partial pressurizations were also performed in order to obtain an hermetic seal.

the ear canal and the air contained within it during tympanometric assessment) is composed of stiffness, mass and resistive elements.

Energy can be stored within the entire ME system in the stiffness and mass elements. This stored energy is referred to as susceptance (B) (imaginary component of admittance). Susceptance values are not absolute, but describe the relationship between the energy stored in the stiffness and mass elements. A positive susceptance (B_s) value is indicative of a system dominated (or controlled) by the stiffness elements. A negative susceptance (B_m) is mass controlled. The complex reciprocal of the susceptance admittance term is reactance (X), this being the energy returned from the system to the probe tip (imaginary component of impedance). Again, this term can be stiffness or mass controlled (X_s or X_m). Negative reactance (X_s) is now stiffness controlled due to the negative sign of the conversion equation[d]. The ME system also conducts energy through it to the inner ear. The flow of energy through the system is termed the conductance (G), this is the real component of admittance. The reciprocal of conductance is resistance (R), the opposition to energy flow through the system. Resistance is the real component of impedance.

Recording of the overall admittance tympanogram is the commonly used clinical procedure. For this study, susceptance and conductance tympanograms were collected separately. Admittance magnitude and phase angle can be calculated from this data. All these tympanograms can then be converted to their impedance equivalents using the mathematical concepts in Appendix B.

6.3 Tympanometric Shape

The 'normal' tympanometric morphology found with adult ears (and some infant ears) at low frequency (226Hz) is a simple bell shape. An example of normal (shaped) low frequency susceptance, conductance and overall admittance tympanograms are shown in Figure 6.1. This data is actually from a term infant. The Jerger[e] system is only used to classify admittance tympanograms at low frequency. This system is not appropriate for classification of susceptance and conductance data at multi-frequencies.

---

[d] Refer to Appendix B for conversion equations.
[e] See Section 3.7.1 for description of the Jerger classification system.

The Vanhuyse[f] system can be implemented for susceptance and conductance (and admittance) data at any frequency. The tympanograms in Figure 6.1 would be of type 1B and 1G (susceptance and conductance tympanograms with one central extrema). The admittance tympanogram is also shown (1Y). Considering susceptance, an adult ear at low frequency would generally show a single peak tympanogram as shown in Figure 6.1. The peak, in this case, is at ambient pressure (0daPa). This is not always the case. Peak location will be discussed later. At ambient pressure (in this case), the tympanogram displays maximum stored energy (positive susceptance value indicating a stiffness controlled system)[g] and maximum conductance for a particular ME system. This peak coincides with the point at which minimum reactance (X) occurs. This is the pressure at which maximum energy enters the ME system.

As pressure in the ear canal is increased (positive or negative) the tympanic membrane (TM) will 'bulge', increasing its stiffness. This increased artificial stiffening of the TM increases its impedance. The TM resists the energy flow into the ME, thus reducing conductance. This applied TM impedance returns more energy back to the probe tip, thus increasing the negative reactance component. More energy being returned results in less energy being stored in the system. When pressure reaches an extreme (-400 or +200daPa) the TM is assumed to be completely stiffened (rigid), thus returning all energy ($X \rightarrow \infty$) with maximum resistance ($R \rightarrow \infty$). This results in both stored energy (susceptance) and conductance equalling zero. This situation is used to compensate tympanograms to eliminate ear canal effects. This will be discussed in more detail in the next section. The basic tympanogram shape is, thus, a direct result of the artificial stiffening of the TM under applied external pressure. The actual morphology of the tympanogram is dependant on the mechanical properties of the particular TM and ME system.

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[f] See Section 3.7.2 for description of the Vanhuyse classification system.

[g] The normal ME system in adults at low frequency (ie. 226Hz) is stiffness controlled and will always result in positive susceptance values. This is not the case in infants and adult ears with pathology.

6.4 Tympanogram Compensation - Ear Canal Effects

When the TM is artificially stiffened, it is assumed that none of the acoustic energy from the probe enters the TM or ME systems. At relatively extreme pressure (-400 or +200daPa)[h], the TM is sufficiently stiffened to act as an acoustically rigid wall. This provides zero admittance with impedance components tending towards infinity. Raw (uncompensated) tympanometric data is a measure of the characteristics in the plane of the probe tip. This includes the first sub-system which is the ear canal and the air contained in it. The ear canal characteristics will obviously change with canal size, tissue properties and placement of the probe tip. It is, thus, useful to eliminate this variable component. Testing at extreme pressure 'detaches' the ME (including the TM) from the readings.

In adults, with a TM stiffened by extreme pressure, the ear canal and TM do act as acoustically rigid bodies. The air contained within the hermetic seal of the probe tip and the TM acts as a stiffness element providing quantity to susceptance, the conductance component being relatively low in the 'normal' adult ear. These values are considered to be purely from the ear canal and are subtracted from later measurements. The low conductance value enables admittance (Y) tympanograms to be compensated directly in adults at low frequency. Compensation of the individual admittance components is necessary for adults at high frequency and infants even at low frequency. This is due to the assumption that the phase angle of sound pressure in the plane of the probe tip and in the plane of the TM are the same. In infants, this assumption is not true, even at low frequency. It has been suggested that the infant ear canal is subject to distention and that the external auditory meatus (EAM) and TM are oedematous[i] in nature and do not act purely as acoustically rigid walls. The interaction of the EAM and TM in infant tympanograms will be discussed later in this chapter. For the data from this current study, each susceptance and conductance tympanogram will be compensated by its individual tail value, this will account for any probe movement between tests.

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[h] For this study tympanograms are compensated at -400daPa.

[i] Oedematous refers to a condition characterized by an excess of watery fluid collecting in the cavities or tissues of the body.

6.5 Notching of Tympanograms

As reported by Vanhuyse *et al.*[12], notching of admittance tympanograms occurs in normal adult ears at high frequency, in adult ears with pathology, and is prevalent in the infant even at low frequency. The lowering in frequency of notch occurrence in infants is due to the ME (including EAM) characteristics. The infant ME is anatomically different and is more susceptible to transient medical conditions. The extent to which infant TM characteristics affect the notching frequency remains uncertain.

It is often reported that notching occurs in susceptance tympanograms when the resistance of the system is larger in magnitude than the negative reactance ($R>X_s$). What does this imply about the mechanical properties of the ear? Considering the 3B Vanhuyse pattern of the susceptance tympanogram in Figure 6.2 (the point of maximum energy flow through the ME system is still located at 0daPa). The central deflection of this tympanogram will correspond to the static stiffness (positive) susceptance of the ME system. When low pressure is applied (positive or negative) the increased artificial stiffness imposed on the TM adds to the total storage of energy of the ME stiffness elements. During the pressure range of the notch, the resistance of the total system is greater than the reactance. This implies that, during this pressure range, the energy lost in frictional elements is larger than the amount of energy returned to the probe tip from the stiffness elements in relation to the mass elements.

At the shoulder of the notch (in fig. 6.2) the resistance and reactance quantities are equal. With increasing pressure to maximum (negative or positive), the increased stiffness (past the optimum for energy storage) of the TM adds to the negative reactance, tending it towards infinity. This leads to zero admittance quantities (for TM and ME components). This implies that the negative reactance is always larger than the system resistance for the single peaked (1B) tympanogram. To examine the frequency dependency of notching in infants we need to look at the resonant characteristics of the total ME system. This will be discussed in the next section.

When the susceptance notch becomes large enough, the ME stiffness is no longer optimum (relative to the mass component). This results in less than optimum conductance. The artificial stiffness of the TM under pressurization then increases the conductance of the overall system (instead of decreasing it), thus notching the

conductance tympanogram (3G). It must be noted that notching always occurs in the susceptance tympanogram first.

6.5.1 Resonance

The ME system is said to be at its resonant frequency when the overall susceptance is equal to zero relative to the tail value (the tympanogram must be compensated, see Appendix B). At high negative ear canal pressure, susceptance is zero with negative reactance tending towards infinity (all energy returned). At resonance, both susceptance and reactance equal zero. This is not to imply that there is zero stored energy in the ME system. It merely implies that energy storage is distributed between the stiffness and mass elements equally. The resonant frequency of any particular mechanical system is dependant on the stiffness and mass component characteristics. In adults, the ME system has a resonant frequency at approximately 1000Hz[1] (ranging from 800Hz to 1200Hz in 'normal' adult ears[2]). Below the resonant frequency the system will display the stiffness elements dominating the storage of energy. Notching will occur when the frequency starts to approach resonance. Just beyond the resonant frequency, the notch will have a negative susceptance, indicative of a mass dominated system.

In infants, the susceptance tympanogram is often reported as being notched even at 226Hz, with susceptance values being negative in some cases, suggesting a mass controlled ME system. This dramatic reduction in resonant frequency between adults and infants is due to a combination of anatomy, transient ME conditions, TM characteristics and possibly EAM properties. These low frequency characteristics suggest that the infant ME system is approaching, is at, or is beyond (in the case of a mass controlled susceptance) the resonant frequency.

As previously stated, the relationship between stiffness and mass elements will control the frequency at which resonance occurs. Whilst the ME system is complex in geometry and properties, it is known that increasing the stiffness elements will increase the resonant frequency. This is true of any mechanical system. Alternatively, the resonant frequency can be lowered by addition to the mass elements. This could imply that stiffness of the ME system in infants (ligaments, tendons, etc.) combine to create a relatively loose mechanical system. Alternatively, it could imply that an increase in mass

(ME bones, etc.) creates the mass dominated system that is often observed (ME fluid could also add to mass elements). The reduced resonant frequency of the infant is probably due to a combination of reduced stiffness and increased mass. However, it is essential to consider the first component of the ME system, the TM. In adults, the TM is a stiffened elastic membrane, its properties are important for energy transfer. Roberts *et al.*[3] reported that in the infant this membrane can suffer from a reduced elasticity (stiffness), and would thus add to the loosening characteristics. They observed the infant TM under applied pressure using pneumatic otoscopy. They also reported that the infant TM can be thickened and dull red in appearance. It is known that tissues in neonates are oedematous. It is, then, possible that the TM could also be oedematous in nature and thus present an additional mass element.

It is possible that the tympanometric characteristics can be totally dominated by the properties of the TM[4]. If the TM characteristics are more dominant than the ME structures, the TM will dominate the data. Impedance measurements are merely measures of the input impedance of a composite system; the portion of the ear canal to the TM and of the ME beyond the TM. A low impedance former system (ie. TM) would make measurement of the ME behind it impossible. It is understandable that the infant ME system would be loose in nature, this reducing the overall stiffness characteristics. The mass elements of the infant ME would be low. However, the presence of fluid or a thickened oedematous TM would increase mass content appreciably.

6.6 The Effect of Successive Runs

Tympanometric recording using multi-frequency probe tones introduces the necessity for a relatively high number of test runs. It is, therefore, possible that some interaction may occur between the number of runs and the various parameters recorded. It has been suggested in the past that dummy sweeps should be performed prior to testing[5]. Even so, there is an expectation that with successive tympanometric runs, changes in complexity and peak admittance values are possible. The complexity of tympanograms can be assessed using the Vanhuyse classification system; Y_1, Y_8 and Y_{28} will be analysed. Peak (or central extrema) location and static compensated immittance values will also be investigated in relation to successive runs.

The negative tail value, used for compensation, has also been reported as being affected by successive runs. The negative tail values for this study will contain variability due to movement of the probe tip. This is especially true when testing the newborn and premature populations, the probe being hand-held due to the lack of support provided by the EAM. This movement is minimised when testing adults due to the more mature structure of the meatus.

Whilst the numerical analysis of basic admittance (Y) tympanograms is clinically unsound (due to the violation of phase angle assumptions[j]), it is valid for assessing changes with successive runs.

6.6.1 Successive Vanhuyse Classifications (Term Data)

The admittance tympanograms were assessed with the Vanhuyse system, these being classified by the number of extrema in the admittance (Y) data. All term subjects displayed Vanhuyse types for admittance recorded at 226Hz. Results for the term group as a whole can be seen in Table 6.1. Tables 6.2a and 6.2b show results for subjects tested within and beyond 3 days after birth respectively. Wilson *et al.*[6] reported increasing complexity of susceptance and conductance tympanograms in adults tested at 226Hz for successive runs. The predominant effect was an increase in the incidence of the 3B pattern over 10 trials. They also reported an increased incidence of the 5B and 3G patterns. Notching of susceptance tympanograms will often result in notching of the admittance tympanogram.

Considering the term group as a whole, it can be seen that the initial admittance recording (Y_1) results in an equal division (50%) between the 1Y and 3Y patterns. By the eighth successive test (Y_8), 68% of subjects display the 3Y pattern. This result is repeated for Y_{28} with 71% having 3Y. This suggests that the major effects of successive tympanometric runs occur within the first eight tests. This supports the conclusion by Wilson *et al.*[6] that successive changes in ear canal pressure will result in more complex tympanograms. They suggested that this may be associated with a looser ME transmission system. They reported that the main effects were found between the 3rd and

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[j] Refer to Appendix B for assumptions regarding phase angle and compensation.

5th successive runs. The Vanhuyse results from this current study indicate that the complexity of tympanometric data may increase over the first eight test runs.

Considering the time after birth of the test session, both groups display an approximately equal division between 1Y and 3Y for the first test, with an increase in the 3Y pattern for the eighth test. However, the last test shows an equal division (1Y/3Y) for subjects tested within 3 days, compared with the 92% of subjects displaying the 3Y pattern for those tested after 3 days. It is not known whether this result is due to subject or medical interaction.

6.6.2 Effects of Trials on Negative Tail Values

Wilson *et al.*[6] also reported on negative tail values for susceptance and conductance at 226Hz. They concluded that there was a predominant increase in negative tail values for both susceptance and conductance over 10 trials. Term data from this study was examined for differences occurring with successive runs for admittance tympanograms at -390daPa[k] for a 226Hz probe tone. Seventy-five percent of subjects show a decrease for the first eight successive runs (Y_8-Y_1)[l], with 58% showing a decrease over the remaining tests (Y_{28}-Y_8). Over the entire test period (Y_{28}-Y_1), 74% of subjects show a decrease. This result does not agree with data from Wilson *et al.*[6]. The decrease in negative tail values for admittance (Y) data suggests some reduction in either susceptance or conductance. It is possible that the probe tip is more deeply inserted during the latter stages of testing, with infants becoming more restless. This is not a problem, as all immittance data is compensated individually, allowing for the movement of probe tip that occurs when testing this population. It was often necessary to re-position the probe in between trials due to subject movement or non co-operation. These results indicate that changes in negative tail value do occur, but that these changes are not characteristic of a successive run effect. The majority of subjects display a decrease with successive runs. The variations that are observed will be eliminated by the compensation procedure.

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[k] -390daPa is used due to -400daPa not always being recorded. This is due to the GSI33 instrument.
[l] Y_8-Y_1 refers to the difference in value between the eighth and the first run.

6.6.3 Peak Pressure Location

The location of the central extrema for the term group was examined for the effect of successive runs. Mean pressure locations for Y_1, Y_8 and Y_{28} are -5.7 ($P_{0.05}$=8.01), -12.9 ($P_{0.05}$=7.40) and -12.5daPa ($P_{0.05}$=7.68) respectively. This suggests that there is, in general, a tendency for the peak location to become more negative over the first eight successive runs. The following 20 runs have little effect. It is not known why this behaviour occurs, but the changes observed are relatively small compared with the variation normally seen with this parameter. Data shows that for Y_1, 54% of the subjects have negative pressure peak locations (32% positive). This percentage figure rises to 71% and 75% for Y_8 and Y_{28} (21% and 13% having positive peak locations respectively). The remainder displayed 0daPa peak locations. This supports the move to more negative pressure peak locations with successive runs, this being most pronounced during the first eight runs. However, the movement of peak location is relatively small compared with the fluctuation commonly seen with this parameter.

6.6.4 Static Peak Immittance

The mean compensated static peak immittance values for Y_1, Y_8 and Y_{28} increase from 0.696 ($P_{0.05}$=0.071) to 0.759 ($P_{0.05}$=0.087) and 0.804mmho ($P_{0.05}$=0.096). The mean difference for the first eight runs (Y_8-Y_1) and the remaining runs (Y_{28}-Y_8) are 0.063 ($P_{0.05}$=0.089) and 0.045mmho ($P_{0.05}$=0.086) respectively. This indicates that the greatest increase is observed for the first eight successive runs. The mean difference for all runs (Y_{28}-Y_1) is 0.114mmho ($P_{0.05}$=0.094). These mean values suggest an increase in Y peak values with successive runs. However, the positive increases account for 61%, 58% and 75% of subjects for Y_8-Y_1, Y_{28}-Y_8 and Y_{28}-Y_1 respectively. It can be seen that 39% of subjects for Y_8-Y_1 actually display a decrease with successive runs. It should be noted that these decreases with successive runs do not correspond to notching of the tympanogram (a change in Vanhuyse classification from 1Y to 3Y). The changes found are relatively low when considering the overall admittance data.

These results indicate that modifications to tympanometric data do occur within the initial runs (Y_8-Y_1). The changes in Vanhuyse classifications affect only 18% of subjects with the induced notching being minor. The changes observed in peak static values and peak location are low. It is proposed that the low variation seen with this data is in part due to the process of obtaining an hermetic seal with this population. The majority of infants actually experience a number of partial and full pressurizations before testing is commenced.

6.7 Tympanogram Morphology

6.7.1 Term Characteristics

At 226Hz, the morphology is relatively standard with 100% of data being classified by the Vanhuyse system. The Vanhuyse system will be discussed in the next section. At higher frequencies the strict implementation of the Vanhuyse system is not possible. The morphology tends to be more complex and non-uniform. A typical set of compensated susceptance, conductance and admittance tympanograms (226-2000Hz) for a term infant can be seen in Figure 6.3. Only a small percentage of the term infants display simple, uniform single peaked data at 678Hz. Approximately 50% of tympanograms at 678Hz display very broad single peaked data which is highly asymmetrical. Positive pressure data tends to show a notch shoulder at the extreme pressure value (+200daPa). Negative pressures create mass susceptance characteristics, these gradually decline to zero at -400daPa. These characteristics can, in some cases, approach a distorted 3B/5B pattern with only two clear extrema. The 3B pattern would account for the positive pressure notch shoulder appearing at +200daPa, with negative pressures being constant or mass orientated. These patterns do not satisfy the Vanhuyse criteria due to the lack of clear notch shoulders (see fig. 6.3, 900Hz susceptance tympanogram). These patterns are indicative of ear canal wall mobility or the presence of oedematous conditions, cerumen or external otitis media (EOM).

At 1000Hz, there is a greater prevalence of single peaked susceptance and conductance data. The susceptance characteristics tend to display asymmetric patterns, the positive pressure values remaining high until maximum pressure. Two of the term

infants display sloping susceptance tympanograms, increasing with positive pressure. Eleven infants (39%) have a broad 1B pattern, their static immittance being of peak susceptance. The remaining 15 infants (54%) have their static immittance described by a trough value, these patterns loosely fitting the distorted 3B pattern. All infants display a conductance pattern resembling 1G with peak locations being variable. In conclusion, 93% of term infants tested at 1000Hz could have their static immittance identified from the tympanogram, nine being mass dominated. It is suggested that the distorted 3B pattern, seen in the majority of subjects, is due to ear canal distention, tissue properties, and possibly some cerumen/EOM interaction.

6.7.2 Preterm Characteristics

The preterm infants at 226Hz display a high percentage of patterns that are standard in nature (19 of 20 test sessions could be classified using the Vanhuyse system). However, there appears to be more variation than with term infants at higher frequencies. It is important when considering the optimum frequency for testing that the static susceptance is stiffness dominated for increased sensitivity. In addition, the morphology needs to be easily interpreted, providing clear extrema for marking.

Considering the preterm infants (with more than two test sessions) tested at 678Hz, the six infants were tested a total of 20 times. Six of these tympanograms did not display a clear extrema for marking. It should be noted that three of these were for subject HARB who produced poor tympanometric data throughout. For the other five infants, identification was 81% successful. The poor morphology is not age dependant, the incidence of poor data being evenly spread across test sessions. It should also be noted that poor data at 1000Hz does not necessarily coincide with the 678Hz results. There is not a problem with the conductance patterns. Extrema are easily identified and morphology is generally more symmetrical than for susceptance. The susceptance tympanograms were assessed for static immittance location morphology, and reported as either peak or trough in characteristic. Of the 14 identifiable tympanograms, 9 display peaks for marking and 5 display troughs. At 1000Hz, ignoring infant HARB, again 81% were identified. The incidences of non-identification are different from those subjects not

identified at 678Hz. Of the thirteen tympanograms identified, six display peaks and seven display troughs.

The morphology of the preterm tympanogram is more complex than those found for the term infants. However, static immittance location is possible even at the higher frequencies. It is necessary to appreciate the distorted nature of the patterns produced.

6.8 Vanhuyse Classification

Tympanometric data was assessed using the Vanhuyse classification system. Data for frequencies between 226 and 678Hz were classified. Tympanometric data above 678Hz was not assessed with the Vanhuyse system due to the lack of conformity. Vanhuyse types 1B1G, 3B1G, 3B3G, 5B3G and OB1G were identified. An 'Other' category was included for data which did not fit the former criteria. This category was necessary for the frequencies above 226Hz. The Vanhuyse criteria was strictly enforced. Vague inflexions in data were not accepted as maxima and minima for this classification system. At higher frequencies broader notching characteristics occurred which did not satisfy the Vanhuyse system. It is important to distinguish between normal shape W-patterns (sharply notched) and pathological broader patterns[7].

Considering the term group as a whole, all 28 infants provided a complete set of susceptance (B) and conductance (G) tympanograms at all frequencies. Vanhuyse classifications for the group are reproduced in Table 6.3. At 226Hz, it can be seen that 86% of subjects display a 3B pattern. This population is equally divided for the 1G and 3G patterns. A relatively small percentage display 1B1G, 5B3G and 0B1G patterns. All data at 226Hz can be identified by the Vanhuyse system (0% 'Other' category). Holte *et al.*[8] found that 98% of term infants could be classified by Vanhuyse in the first week after birth. Meyer *et al.*[9] reported that their case study infant displayed, in the first four weeks after birth, a 3B1G pattern with a 3Y. This changed to a 1Y (with 3B1G) after four weeks. Keith[10] reported the 3B1G1Y pattern in only 18% of neonates soon after birth. For this current study, 61% display a 3Y calculated admittance pattern. There are only two cases of 3B1G3Y, these being the only patterns where G and Y extrema are not equal. The 3B1G1Y pattern is found in 36% of infants.

At 300Hz, there is a greater prevalence of the 3B3G pattern (68%). The dominant feature at this frequency is the 3B pattern (82%), the 3G pattern occurring in 79% of subjects. At this frequency, no subjects displayed the single peaked pattern (1B1G); the 'Other' category accounted for 7%.

Testing at 400Hz would appear to be the limit of usefulness for the Vanhuyse system with this population, 68% of subjects being classified. Virtually all subjects classified by the system (94%) displayed the 3G pattern (65% of the population). The 3B pattern is slightly more prevalent than the 5B (58% of the Vanhuyse classified subjects having 3B, 42% 5B). At 400Hz, Holte *et al.*[8] reported that 5B3G and 'Other' were the most common classifications in neonates tested 1-7 days after birth. In this current study, for data beyond 400Hz (ie. 500, 600 and 678Hz), the 'Other' category accounts for over 80% of all subjects in this population. In comparison, Margolis *et al.*[11] reported 3B3G patterns or simpler in 100% of the adult population at 450Hz. Holte *et al.*[8] reported that the majority of the tympanograms recorded at 710Hz and 900Hz within the first 3 weeks after birth could be Vanhuyse classified, with zero classification at 900Hz within the first week.

It is generally reported that the tympanometric patterns become more complex with increasing frequency[11]. The results from this study would agree with this observation. However, Holte *et al.*[8] suggested an exception from the characteristic, observing more complex patterns at 355Hz and 450Hz than surrounding frequencies. They suggested that this is consistent with the presence of a resonant frequency at 450Hz. Resonant frequency assessment for this study would indicate that 14 infants (50%) display resonant characteristics <226Hz. This would imply that 226Hz (and 300Hz) patterns should be more complex than >300Hz. This is not the case.

The group was also assessed for the time after birth of the test session. Thirteen subjects were tested within 3 days after birth, the remaining 15 being tested after 3 days but within 6 days. Vanhuyse classifications for these groups can be seen in Tables 6.4a and 6.4b. Similar results for both groups are found for 300Hz and above. At 226Hz, the 3B1G pattern is equally prevalent in both groups. However, the most complex pattern for the older subjects is the 3B3G pattern. For the younger subjects 5B and 0B patterns

are also observed[m]. This could suggest that the susceptance (B) data is more variable in infants tested within the first 3 days after birth.

6.8.1 The Preterm Vanhuyse

Eight of the preterm infants were tested for tympanometric data in conjunction with Auditory brainstem response (ABR) testing. A full set of tympanometric data was obtained from all infants tested. The earliest tests were performed at $29^{+5\ n}$ weeks PCA. Fortnightly time categories will be used for analysis to eliminate multiple subject recordings within time categories. There is no Vanhuyse classified data (100% 'Other') for frequencies above and including 500Hz. In comparison, the term data displayed 14-18% Vanhuyse classified data for 500Hz and above (600 and 678Hz). Results for 226, 300 and 400Hz for the various time categories can be seen in Tables 6.5a to 6.5c.

Considering 226Hz, it is immediately obvious that the 1B1G pattern is eliminated from the 226Hz tympanograms (7% for term data). The term data showed that the majority of subjects display 3B1G and 3B3G patterns (these being equally divided). It can be seen in the preterm data that there is a greater prevalence of the 3G rather than the 1G pattern. The 'Other' classification is present in a number of the time categories. It is observed that the majority of subjects in all time categories (apart from 28-30PCA) are of the 3B3G pattern. However, there is a greater percentage of subjects displaying the 5B pattern than in the term group. Again, admittance extrema follow the conductance extrema, the 3G3Y pattern being most prevalent. There is no apparent maturation from the 3Y to the 1Y pattern. Vanhuyse *et al.*[12] suggested that a 3Y pattern would be observed initially (indicative of a predominantly mass controlled system), the later appearing 1Y pattern showing a lesser degree of mass influence. Meyer *et al.*[9] reported that the 3B (1G1Y) pattern will continue until 41 days old (≈6 weeks). From 51 days (≈7 weeks) a 1B pattern will be observed. A number of preterm infants show 5B patterns on initial testing, these changing to 3B by the next session. However, the 5B pattern does occur at later PCAs.

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[m] The subjects displaying the 5B3G and 0B1G patterns, infants MOR and BYE respectively, were tested at 3 days after birth.
[n] The value in superscript denotes additional days of PCA.

At 300Hz, there are no 1B1G or 3B1G patterns for any of the time categories. This eliminates the single peaked tympanograms 1B and 1G. The absence of 1B1G is also found with the term data. However, there is some evidence of the 1G pattern in combination with the 3B for the term group. Considering the 3G tympanograms, the accompanying susceptance shows a division between 3B and 5B in favour of the more complex 5B. At 400Hz, for the time period 28 to 32 weeks PCA, 100% of patterns fit the 'Other' category. For the later time periods (>32 weeks PCA), 40% or more of the patterns in each age category are of the 'Other' classification. The remainder of the subjects display a 3G pattern with the majority having a 5B rather than a 3B pattern from 34 weeks PCA onward. The term group favoured the 3B pattern.

In summary, at 226Hz, 86% of term infants display the 3B pattern. The accompanying conductance tympanograms (with the 3B patterns) are equally divided between 1G and 3G. There is a low incidence of the 1B1G combination. This pattern is eliminated in the preterm infants even at 226Hz. Preterm patterns tend to be more complex with a greater prevalence of the 5B pattern. The 3G (rather than 1G) pattern is also more prevalent.

At 300Hz, 80% of term infants display the 3B pattern with 79% displaying the 3G pattern. There are no 1B or 1G patterns. The preterm infants display a greater prevalence of the 5B over the 3B pattern. These patterns are accompanied by the 3G pattern. At 400Hz, 68% of term patterns can be classified (not 'Other'). Of those classified, 58% display 3B (42% 5B) with 94% showing the 3G pattern. In the preterm infant, 100% of patterns are classified as 'Other' before 32 weeks PCA. After 32 weeks, 40% or more patterns are classified as 'Other'. The remaining infants show a tendency for the 5B pattern, all have an accompanying 3G pattern. This shows a high incidence of the 3G pattern in both term and preterm infants. However, the occurrence of the 5B pattern is much greater in the preterm group. Above 400Hz, less than 20% are identified for the term group with no classifications (100% 'Other') for the preterm group.

6.9 External Auditory Meatus (EAM) Effects

The first sub-system, when considering tympanometry, consists of the volume of air contained in the EAM. This volume depends on EAM size and the depth of probe insertion. The dimensions and properties of the adult EAM means that this air volume can be represented as a pure acoustic stiffness element. This element cyclically stores and releases the energy without dissipation[13]. At extreme pressure, with the ear canal and TM acoustically rigid, a positive susceptance value will be observed. This is indicative of the stiffness supplied by the air volume. A small fraction of the energy within the EAM will be dissipated as a result of friction within the medium and surrounding surfaces. This will give rise to a low conductance value.

In infants, it is well reported that the ear canal is more compliant and distensible[14]. The adult EAM is surrounded by bone on its inner third. This osseous portion of the ear canal is not completely formed until approximately one year of age[15]. Holte *et al.*[8] used pneumatic otoscopy to observe movement of ear canal walls with pressures similar to those used in tympanometry. Positive and negative pressure pulses of 250-300daPa resulted in ear canal diameter changes of up to 70% in 1 to 5 day old infants. However, some ears in their study showed <10% diameter change, even in the first week after birth. They stipulated that beyond 31 days after birth, ear canal wall distention does not exceed 10%. Beyond 56 days there is no movement of the osseous portion. It is, therefore, clear that a certain amount of movement of the ear canal is present in the neonate. The change in ear canal diameter will introduce a variable stiffness component. Generally, as a volume of air reduces it becomes less compliant (increase in stiffness).

It is, thus, appropriate when dealing with neonates to examine the EAM as a varying mechanical system. Sprague *et al.*[14] highlighted the difficulty of distinguishing contributions of ear canal resonance from resonance characteristics of the ME. This is due to the distendable nature of the ear canal, not functioning as a hard-walled cavity°. Holte *et al.*[8] suggested that no ear canal diameter change is found in infants from approximately one month of age. They inferred this to mean that the ear canal will

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° The mathematical concepts of tympanometry assume that the ear canal acts as an acoustically and mechanically rigid structure.

function as a hard-walled cavity. A lack of overall movement of the ear canal, however, does not confirm that the surface tissues will act as acoustically rigid structures as assumed in the adult. It is possible that oedematous tissues will vibrate and absorb energy. This would add to the conductive component. The susceptance can also be increased by the oedematous condition of the EAM in the neonate, these tissues vibrating. The neonate ear canal can thus alter impedance measurements in two ways; changing its volume under pressure application and the vibratory motion of the surface tissues.

6.9.1 Effect of Ear Canal Mobility on Tympanometric Data

The variety of neonate tympanometric shapes have been reported as being due to ear canal distensibility[16]. However, Holte *et al.*[8] suggested that this is generally not the case. They found multiple peak tympanograms at 226Hz in infants without visible ear canal distention. They also found no correlation between 226Hz tympanogram complexity and ear canal mobility. The most obvious indicator of ear canal mobility is a monotonic rise in stiffness susceptance with increasing pressure from negative to positive, this resulting in a 0B1G pattern. This can also be produced by probe tip movement. This pattern was found in one of the term infants and in one preterm infant (on this current study) tested at 6 days after birth. As the air volume increases, it also increases its compliance, storing more energy as stiffness susceptance. Whilst the 0B1G pattern is not widespread, it is possible that some interaction is present with other patterns at extreme pressure values. The rise in tail values between extreme negative and positive pressure (producing asymmetry in tympanograms) suggests some interaction. The ME characteristics will contribute to the central low pressure region of the tympanogram.

6.9.2 Effect of Ear Canal Acoustic Properties

The asymmetric extreme pressure characteristics can also be found in subjects without ear canal mobility. Holte *et al.*[8] suggested that vibratory motion of ear canal walls could possibly add a conductive component. In adults, where ear canal tissues are

acoustically rigid, the resistance component of the ear canal will be large ($R \rightarrow \infty$), resulting in zero energy absorption (low conductance). With oedematous tissues, the resistive component is reduced and energy absorbed. The movement of these tissues also affects the susceptance data.

Similar tympanometric characteristics are observed with material in the EAM, such as external otitis media (EOM) or soft cerumen. The effects at higher frequencies can clearly be seen. Figures 6.5 and 6.6 show tympanogram characteristics for EOM and soft cerumen respectively. Pappas and Wolcott[17] show three examples of 660Hz tympanogram characteristic of EOM (see fig. 6.5). These display the increase in susceptance at positive pressure, leading to an asymmetric pattern. In each case the peak is a central minima. The susceptance tympanogram in Figure $6.5_{(a)}$ shows the notch shoulders to be absent. Figures $6.5_{(b)}$ and $6.5_{(c)}$ display relatively constant susceptance values at pressures beyond the notch shoulders. There is also the characteristic of a less obvious, and lower valued notch shoulder, on the negative pressure side.

The Feldman[18] data (see fig. 6.6) shows 220Hz and 660Hz tympanograms before and after removal of soft cerumen from the ear canal walls. The cerumen causes a slight 3B pattern to appear in the B_{220}[p] tympanogram. The 3B pattern introduced at 660Hz is more pronounced (dramatically reducing static immittance when compared to the negative pressure tail). There is also the asymmetrical susceptance tail values. There would seem to be little effect on G_{220}, with G_{660} having an increased static peak value. The most obvious effect is the vertical shift of the G_{660} baseline (tail values). Considering the negative tail phase angles[q], this data suggests that whilst negative tail phase angle at 220Hz remains >80°, the 660Hz negative tail phase angle is significantly reduced (≈60°) with the presence of cerumen (>70° after removal). This suggests a loss of hard-walled characteristics at negative pressure. Feldman[18] noted that the system in Figure 6.6 is flaccid and the shape is suggestive of an eardrum abnormality.

It is of interest to examine the numerical data of Feldman[18] to establish the effect of EAM characteristics to tail and static peak values. Numerical data taken from Figure

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[p] B_{220}, G_{660} refer to susceptance and conductance tympanograms recorded with a 220Hz and 660Hz probe tone frequency respectively.

[q] The phase angle describes the relationship between susceptance and conductance. For the EAM behaving as a hard-walled cylinder the susceptance is much greater than the conductance. This is due to the volume of air in the EAM only having a stiffness component (no overall conductance). If the EAM is not acting as a hard-walled cylinder then a conductance component is introduced. This reduces the phase angle considerably.

6.6 can be seen in Table 6.6. In this case the pressure range is from -200 to +200daPa. It can be seen that the reading taken for B_{220} at peak is unaffected by the cerumen. It is the tail values which are increased, equally for both positive and negative extreme pressures. This leads to a decrease for the compensated peak value. The G_{220} tympanogram shows that both tail and peak values are unaffected. At 660Hz, the effects are more exaggerated. Considering B_{660}, the tail values are again increased by the cerumen, the positive tail being more affected. Unlike B_{220}, the peak value (B_s) is considerably reduced. With negative tail compensation, the compensated peak value decreases to a lesser extent with cerumen present. The peak G_{660} value is also affected, rising considerably with cerumen present. There is also a symmetrical increase in tail values and an increase in the compensated peak value.

These results indicate that at 220Hz the ear canal stored energy (B_s) is increased by cerumen. At 660Hz, this increase in stored energy (B_s) is greater with positive pressure in the ear canal. The ear canal also conducts (absorbs) more energy at 660Hz with cerumen present, this being equal for negative and positive pressures. Whilst static peak values are unaffected at 220Hz, both susceptance and conductance are affected by the presence of cerumen at 660Hz. B_{660} and G_{660} peak values show that conductance (G) is greatly increased and stored energy (B_s) is much reduced. These characteristics are still displayed with compensated data. Whilst this shows the ear canal data is not always affected in the same way as the static peak data, the ear canal effects from cerumen are not large enough to disguise the peak characteristics.

Tympanometry is ideally suited to monitoring changing conditions with treatment and time. With one off tests, the asymmetrical tail values should not be ignored. The susceptance tail discrepancy (at 660Hz) with cerumen was 1.1mmho. The value is only 0.3mmho after removal. This, in combination with a highly conductive system, at both peak and tail pressures, would seem to be indicative of cerumen presence. The degree of conductivity is best identified by the negative tail phase angle which reduces from 75° to 59° for cerumen presence. This situation is not present at 220Hz, conductance values tending to zero.

6.9.3. Ear Canal Measurements

The low incidence of the 0B1G pattern suggests that excessive ear canal wall distention is not a problem with either term or preterm infants. However, they readily show similar characteristics to those found with cerumen or EOM. Ears were visibly checked for excessive cerumen and the probe tip observed after testing for cerumen presence and partial blockage. This was not found to be a problem. It is suggested that cerumen and oedematous tissue conditions could cause similar tympanometric characteristics.

Mean negative tail values for term infants (fig. 6.7) suggest that susceptance is greater than conductance (at -400daPa) up to 400Hz. The best results are gained for 300Hz, where 32% of term infants display phase angles >70° for the negative tail (see fig. 6.9). Tail phase angles should be >80° for accurate ear canal measurement. At all other frequencies this value is much reduced; no phase angles (negative tail) were >70° beyond 678Hz. The mean plot suggests negative tail conductance values to be greater than susceptance beyond 400Hz. This results in low phase angles. At positive pressure (+200daPa), susceptance is greater than conductance up to 678Hz (fig. 6.8). Again, few infants have phase angles >70° (32% at 226Hz), none being >70° above 300Hz. Mean data for symmetry of tail values show an approximately linear increase with frequency for both susceptance and conductance. The relationship between susceptance and conductance is relatively constant. The asymmetry in tail values is greater for susceptance up to 1500Hz.

The mean phase angles for the term infants, for positive and negative tail pressures, can be seen in Figure 6.9. These results show the violation of the compensation assumptions previously mentioned. In adults, it is common to achieve tail phase angles >80°. This indicates that the stiffness susceptance is much greater than the conductance. This behaviour is expected with an ear canal acting as a hard-walled cylinder with only a stiffness element. Negative tail phase angles range from 40° to 70° for the term infants at 226Hz. This reduces to a low at 1000Hz with a range of 6° to 25°.

Considering 226Hz, the preterm group show increasing trends for B_{-ve}[r] with age. There is a less consistent decrease in B_{+ve}. The B_{-ve} data approaches term values, with the majority having higher values than term means in the later test sessions. B_{+ve} data all approach the term mean with age. The discrepancy between B_{+ve} and B_{-ve} (symmetry) also approaches the term mean, the majority having comparable values in the later test sessions. There is a similar relationship for conductance, with G_{-ve} increasing and G_{+ve} reducing (values comparable by third test session). The tail discrepancies are seen to decrease in all infants, values being comparable to the term mean by the third or forth test session (beyond 30 days after birth). Data at 678Hz show similar characteristics to those recorded at 226Hz. However, there are more trend inconsistencies. The positive-negative tail discrepancy for susceptance decreases to term values by the second test session in all infants, with a continuing decreasing trend thereafter. Conductance discrepancies are term-like by the second or third test session, again with a continuing trend.

These results suggest that the discrepancies observed between positive and negative tails for both susceptance and conductance will reduce due to the behaviour of both tail measurements. It is also evident that the preterm infants display term-like values (during the preterm period) which continue to decrease with age. This indicates a change in the EAM component with age. It is not possible to identify whether this alteration is due to acoustic or mechanical modifications to the EAM.

6.10 Compensated Static Peak Immittance

All tympanograms (226-2000Hz) were analyzed for compensated peak static immittance. Due to the less clear tympanogram peaks in data collected above 400Hz, as evident in the discussion of Vanhuyse results, peaks were identified from the 226Hz and 300Hz tympanogram data. These pressure peak locations were then used to assess immittance values for the higher frequency data. The ambient pressure (0daPa) data was not used due to the low incidence of peak location at this pressure in the low frequency data. Peak location at low frequency may not necessarily coincide with peak locations at

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[r] B_{-ve} and G_{+ve} refer to susceptance and conductance tympanograms at negative and positive tail pressures respectively (-400daPa and +200daPa respectively).

higher frequencies. However, the reliability of peaks at higher frequencies is not sufficient to allow successful identification of peak locations. It is anticipated that useful characteristics will be found at higher frequencies using this method. Data is plotted against frequency for each subject. These plots replicate the information that would have been obtained by inclusion of the sweep frequency routine from the GSI33 instrument. Completion of this routine was not possible as previously stated. This is due to the level of subject co-operation required for the test[s]. The static data at the tympanogram peak is a representation of the ME characteristics. This occurs at the point of maximum energy admittance to the system.

6.10.1 Pressure Peak Location

Susceptance and conductance tympanograms (226Hz) were assessed for peak location. Central deflections were used (where notching was present) with reference to the point of minimum negative reactance (X_s) (in the case of a stiffness controlled system). Seventy-nine percent of term infants display negative pressure peak location for susceptance (others showed positive or zero), with values ranging from -50 to +20daPa. The mean is -19.6daPa ($P_{0.05}$=6.3). Conductance tympanograms also show a negative bias, 68% displaying negative peaks. The range is the same as for the susceptance data. The mean is -15.4daPa ($P_{0.05}$=6.5). The calculated admittance (Y) data shows a range of -60 to +20daPa with a mean of -17.9daPa ($P_{0.05}$=6.6). Negative admittance peak location is displayed in 71% of subjects.

The existence of a transient negative pressure is characteristic of the infant population. For interpretation of data it is necessary to investigate the degree of negativity which is considered non-pathological. Brooks[19] identified a range between ambient (0daPa) and -170mm H_2O[t] in 95% of paediatric ears tested. Porter[20] suggested that a ±50daPa range was applicable for normal children and adults. Other studies[21, 22] found lesser ranges to be appropriate (±25 or ±30daPa) for the adult population. Considering the neonate population, the range between -50 and +20daPa would seem to

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[s] The sweep frequency routine on the GSI33 instrument requires several procedures to be performed without loss of the hermetic seal. The complete process takes approximately 1 minute. This was found to be impractical with restless infants.

[t] 1.02mm H_2O=1.00daPa

be acceptable. It should be remembered that peak location is the most unstable parameter in tympanometry. As such, inferring pathologies for mild pressure deviations (±40daPa) is inappropriate.

Negative pressure is often accounted for by poor eustachian tube function or ME effusion. It is not possible to identify the actual cause of the mild negative pressure characteristic in the infant population, only that the mild variations are appropriate for this population. From a clinical point of view, the negative pressure in neonates should be considered as abnormal but not pathological.

Observing preterm data, the variability of peak location does not show any clear maturational characteristics. The most pronounced trend is the change in peak location for susceptance (at 226Hz). Six of the eight infants tested show a positive trend (peak moving in a positive pressure direction with age). The range for the susceptance peak is -50 to +30daPa, this being comparative with the term infants. With the fluctuating nature of this parameter it is not appropriate to engage a detailed age analysis. Examining conductance data at 226Hz, four of the eight infants display the positive trend with age (range of ±40daPa, with one subject at +70daPa). This result is repeated with both the calculated admittance (Y) data and that recorded at the beginning of each tympanometric test session (Y_1).

These results indicate that peak location varies between subjects and that there is a negative bias. However, the incidence of extreme pressure location is minimal.

6.11 Term Susceptance - Resonance Characteristics

As previously stated, the sweep frequency can be used to assess the variation of static peak immittance with frequency. This variation can indicate the interaction of stiffness and mass elements. This allows identification of the resonant frequency of the system. This is important information for assessing any mechanical system. Considering the term data, the plot of mean immittance data (B, G and Y) versus frequency can be seen in Figure 6.10. At 226Hz, mean susceptance is positive. This suggests that, even though the majority of tympanograms are of a 3B nature, the notch is not negative in value when compared with the negative pressure tail value. Mean susceptance at 300Hz is negative suggesting a mass controlled system. At 400Hz, the mean is again positive

(stiffness controlled). This indicates resonant frequency characteristics for the majority of infants within this frequency range.

Analyzing whether the system is stiffness or mass controlled can be done by assessing the sign of the peak susceptance value in relation to the negative tail value. Table 6.7 shows the percentage of subjects displaying positive and negative susceptance for varying frequency. At 226Hz, the term group is equally divided, 50% being positive (stiffness controlled) and 50% negative (mass controlled). At 300Hz, there is a prevalence for mass control (82% having negative susceptance). The susceptance is again equally divided at 400Hz. This suggests that the majority of term infants will have mass controlled characteristics at some point between 226 and 400Hz (actually 93%). Between 500 and 1000Hz, there is a clear prevalence for positive susceptance (percentages being approximately 90% throughout). Frequencies of 1500Hz and above display an increasing trend for a return to negative susceptance.

Resonant characteristics are, therefore, suggested between 226 and 400Hz for the term neonate. This dip towards a mass dominated system at 226/300Hz suggests a resonant frequency of approximately 300Hz. Holte *et al.*[8] studied the maturation of tympanogram characteristics from the first month after birth onwards in 23 term infants. They found a dip towards mass reactance between 450Hz and 750Hz in 16 of the 43 ears tested in this group. They concluded that there is a possible resonant frequency at 450Hz in the youngest group. Meyer *et al.*[9], who studied a single term infant from birth onwards, reported resonant frequency characteristics between 300 and 400Hz for the first 60 days after birth.

Examining the resonant frequencies identified from the susceptance data for this current study, 50% show a resonance <226Hz, 39% have resonance between 226 and 300Hz, with 4% displaying resonance between 300 and 400Hz. Seven percent of the term study showed no resonance characteristics before 1000Hz. It is, therefore, clear that ≈90% of the term neonate population tested for this study will show mass dominated characteristics at some frequency at, or below, 300Hz.

It has previously been reported that pathologies such as ossicular discontinuity, which result in the ME acting as an acoustic mass, show poor sensitivity to tympanometric assessment[23]. The routine use of a 226Hz probe tone is acceptable in the majority of adults where the ME system is stiffness dominated. Tympanometry is the

most sensitive for a stiffness dominated system. With the term neonate (in this current study) 50% of infants have mass controlled ME systems at 226Hz. This suggests that tympanometric assessment at 226Hz with this population will provide poor sensitivity for tympanometric data. As Meyer *et al.*[9] found, the neonatal ear matures from a mass controlled to a stiffness controlled system at low frequency. Their results, from a single infant, suggested that a stiffness controlled ME system is found after 100 days after birth. The results of this current study lead to the conclusion that low frequency tympanometry will lack sensitivity for assessment of ME function in 50% of term neonates tested soon after birth. Numerical data at 226Hz gives a mean of 0.039mmho (S.D. 0.314).

Data at 678Hz shows that 97% of term infants display positive susceptance data. At 1000Hz, 80% of infants continue to show positive susceptance. This indicates a more prevalent stiffness controlled region at higher frequencies. The susceptance means are 0.399 (S.D. 0.209) and 0.396mmho (S.D. 0.497) for 678 and 1000Hz respectively. This could indicate that a higher frequency is more sensitive for tympanometric assessment with this population. This will be examined further in the discussion chapter.

6.12 Preterm Susceptance - Maturational Characteristics

The maturational characteristics of preterm susceptance data is of interest. The changes can indicate alterations in ME function that can modify the resonant characteristics. These characteristics are important for 'normal' ME mechanical function. Analysis will concentrate on the standard test frequencies; 226, 678 and 1000Hz. Table 6.8 contains birth and test information for the preterm infants referred to in this section. Mean immittance data (the means are of all the data collected from the preterm infants) is presented in Figure 6.11 to aid comparison with the term group. This will highlight any differences between the two populations. Figures 6.12 to 6.14 show individual preterm susceptance data for 226, 678 and 1000Hz probe tones.

6.12.1 226Hz Probe Tone

Infants DERO, HARA, HARB and NAIL show reasonably consistent susceptance values between initial test (7-8 days) and the second test session (19-24 days). Subject NAIL is stiffness controlled, the younger infants being mass controlled. It is noticeable that all the preterm infants post 20 days show an increase in mass contribution, this generally being dramatic. Of those infants tested post 40 days (4 infants), 50% then display a decrease in B_m. The two preterm infants that display B_s characteristics at approximately 20 days after birth (JARA and NAIL) both become mass controlled before 30-36 days. It is, therefore, clear that there is a difference between term and preterm susceptance characteristics at 226Hz. Beyond 20 days after birth, all preterm susceptance data is mass controlled. The term infants are equally divided between stiffness and mass dominance. The mean susceptance at 226Hz from all data collected for the preterm infants is -0.183mmho (S.D. 0.224).

Identification of resonant frequency characteristics show that infants DERO, HARA, HARB and JARB all show resonance <226Hz throughout testing. NAIL and JARA initially show resonance between 226 and 300Hz. Both subjects reduce to <226Hz by 30 and 36 days after birth respectively.

6.12.2 678Hz Probe Tone

All three of the early GA infants (DERO, HARA, HARB), tested 7-8 days after birth, display positive susceptance values. This suggests a stiffness controlled system. NAIL, tested at 7 days, also shows a positive susceptance. Whilst DERO, HARA and NAIL continue to be stiffness controlled throughout the preterm period, HARB fluctuates between stiffness and mass control. JARA and JARB are both stiffness controlled at 21-23 days. By 36-37 days JARA has become mass controlled (returning to stiffness control by 51 days). JARB shows a decline into mass control by 50 days. The incidence of mass controlled systems is much greater than seen with the term infants, 96% of term infants are stiffness controlled at 678Hz. This is reflected in the lower mean for the preterm group of 0.110mmho (S.D. 0.180).

6.12.3 1000Hz Probe Tone

The variation in susceptance values is greater at 1000Hz. Two of the three infants tested before 30 weeks PCA display a slight mass dominance. Beyond the 7-8 day test, all these infants display positive susceptance. The mass controlled infants show much greater stiffness increases than the infant having stiffness dominance from the first test. Subject NAIL is stiffness controlled throughout and shows similar characteristics as the stiffness controlled infant tested before 30 weeks PCA. Infants JARA and JARB both display similar positive susceptance values at 50-51 days (JARB reducing from a highly stiffness controlled system, JARA maturing from a mass controlled system). It is interesting to note that no infant shows mass control after their first test session. However, JARA was first tested at 21 days after birth and presents mass controlled characteristics. Again, the preterm mean is lower than the term value at 0.339mmho (S.D. 0.315).

6.13 Conductance (and Admittance) Characteristics

Mean conductance and admittance data for term and preterm infants can be seen in Figures 6.10 and 6.11 respectively. The admittance phase angle means are presented in Figures 6.15 and 6.16. Means for conductance show lesser values for preterm, than term data, for frequencies below 1750Hz. Considering the standard test frequencies, the discrepancies are 0.253, 0.272 and 0.581mmho for 226, 678 and 1000Hz respectively. This behaviour is reflected in the calculated admittance data. This indicates that the preterm ME system is admitting less energy through its conductive elements than the term ME system. This behaviour is not due to dimensional or acoustic properties of the EAM, the compensation procedure eliminates these effects. It is, therefore, suggested that these results are due to differences in TM or ME properties, or to dimensional differences of the ME system. It is not possible to identify a particular characteristic with the data available.

The peak admittance phase angle shows the relationship between susceptance and conductance magnitude. Figures 6.15 and 6.16 show a clear difference between term and preterm data. The preterm data shows mean phase angles to be negative below 678Hz.

The term data shows a negative mean at 300Hz only (in the low frequency range). The higher frequency data suggests a relatively constant positive phase angle from 800Hz for the preterm group. The term data shows mass dominance for the mean beyond 1250Hz.

6.13.1 Maturational Characteristics

Maturational conductance trends (226, 678 and 1000Hz) for the preterm infants can be seen in Figures 6.17 to 6.19. The trend in conductance data at 226Hz is divided for the preterm group. The three infants tested before 30 weeks PCA show overall increases in conductance during the preterm period. The remaining infants display a clear trend for decreasing conductance. This suggests that the early born preterm infants could have different ME characteristics.

The increase in conductance data observed for early GA infants at 226Hz is not present at 678Hz. All infants (apart from HARB) display a reduction with PCA. Trends at 1000Hz show more variability. It should be noted that the negative conductance values for 678 and 1000Hz suggest a degree of error. These negative characteristics are caused by the more complex shape of conductance tympanograms at these frequencies. These negative values do not occur at 226Hz, or in any of the term data. This indicates a greater amount of variability for preterm data.

6.14 Preterm Results during the Term Period

For tympanometric assessment to be sensitive, it is beneficial to test the ME system when it is stiffness dominated (ie. when susceptance is positive). It is, therefore, of interest to look at data collected from the preterm group during the term period (36-40 weeks PCA), when the majority of audiological testing would take place. Seven preterm infants were tested within this time period. At 226 Hz, five infants show mass control and two display approximately zero susceptance (suggesting resonance). These results, based on the stipulation of a stiffness controlled system, suggest that 226Hz is not appropriate for testing of preterm infants, even by the term period. In comparison, the term group showed 50% of infants having stiffness dominance at 226Hz. At 678Hz,

three infants are mass controlled, the rest show clear stiffness characteristics[n]. At 1000Hz, 100% of preterm infants have stiffness controlled systems.

The data from these particular preterm infants suggest that a higher probe frequency is beneficial in achieving stiffness controlled characteristics for testing. However, the incidence of negative conductance values at the higher frequencies (678 and 1000Hz) and the more complex patterns are of concern. Care must be taken not to ignore morphology when considering static immittance values.

6.15 Impedance Data - The X-R Relationship

So far, the complex admittance components have been discussed. Tympanometric data for these measurements are produced by the GSI33 instrument. This data can be converted into the impedance components using the conversion equations in Appendix B. An example of impedance data (with its corresponding admittance data) at 226Hz can be seen in Figures 6.20a/b. It can be seen that as admittance components approach zero, the impedance components tend to infinity. Fluctuations around the zero value create the variable data seen with the resistance data between -300 and -400daPa in Figure 6.20b. The reactance data in Figure 6.20b is negative due to the conversion equation in Appendix B.

It is commonly reported that notching in susceptance tympanograms occurs when the resistive elements are greater than the negative reactance (stiffness). Himelfarb *et al.*[25] reported the relationship of R and -X (X_s) for neonates tested with a 220Hz probe tone. Their results are reproduced in Figure 6.21. Whilst there is a gap in data between neonates and infants of 2-4 months of age, a reversal in the X-R relationship is suggested at approximately 1000 hours (41.6 days) after birth.

The term infants for this current study were tested within the first 100 hours after birth. Mean data can be seen in Figure 6.22. The reactance data has been inverted to show the relationship between R and -X. The 226Hz mean reactance is -0.084kohm ($P_{0.05}$=0.222) with a resistance mean of 0.608kohm ($P_{0.05}$=0.052). At this frequency only 14.3% (4 of 28 infants) display a negative reactance which is greater than the resistance component. No notching occurs with this behaviour. This supports the data of Himelfarb

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[n] It should be noted that two infants change their controlling characteristics during the term period

et al.[25], the resistance means being higher than the negative reactance before 1000 hours after birth. At 678Hz, 67.9% of infants display -X>R with means of -0.760kohm ($P_{0.05}$=0.191) and 0.669kohm ($P_{0.05}$=0.098) for reactance and resistance respectively. At 1000Hz this value falls to 46.4%, with means reducing to -0.308kohm ($P_{0.05}$=0.186) and 0.399kohm ($P_{0.05}$=0.048) for reactance and resistance respectively. The mean frequency for the reversal of the X-R relationship (giving -X>R) with this population is 520Hz ($P_{0.05}$=78.3). Four infants show no reversal of the X-R relationship over the entire frequency range (226-2000Hz). These results suggest that 678Hz is the best frequency for testing (of the three standard test frequencies; 226, 678 and 1000Hz).

6.15.1 The Preterm X-R Relationship

The reversal in the X-R relationship (low frequency) at 1000 hours (41.6 days) after birth, as described by Himelfarb *et al.*[25], would not be anticipated in the preterm data. All the preterm infants tested showed continuation of notched susceptance tympanograms throughout the preterm period. This indicates that the resistance is greater than negative reactance throughout the preterm period. There is a suggestion, from the Himelfarb *et al.*[25] data, that the difference between R and -X will increase with age up to 100 hours after birth in the term infant. After 100 hours a reduction with age should be seen. The results of the preterm infants tested for this study suggest that this is not the case for the preterm population. The maturational characteristics for the impedance components will follow the same pattern as the admittance data due to the direct nature of the relationship. It should be noted that the impedance data shows greater variation than admittance data, this being due to the non-linear conversion equations. This reduces the usefulness of impedance data with the preterm population.

6.16 Results Summary

- There are some cases of tympanogram modification with successive runs. Eighteen percent of term infants show a modification to the 3Y Vanhuyse pattern (from 1Y) over the first eight runs. The changes in tail and static peak values are minor.
- Term tympanogram morphology is standard at 226Hz. At 1000Hz, static immittance location (inflexions) can be located in 93% of infants. These patterns tend to be distorted, the peak needs to be confirmed with patterns at lower frequencies.
- Preterm tympanogram morphology tends to be less standard at all frequencies. However, static peak immittance location (inflexions) is possible.
- For term infants, 100% of tympanograms at 226Hz can be classified by the Vanhuyse system. The limit of usefulness for the Vanhuyse system is 400Hz where only 68% can be classified (80% 'Other' above 400Hz). Eighty-six percent of term infants display a 3B pattern at 226Hz, with the accompanying conductance pattern being equally divided (1G/3G). Patterns become more complex with increasing frequency. The 3G pattern becomes more prevalent above 226Hz. The 5B pattern is found in 29% of infants at 400Hz.
- The simple 1B and 1G patterns are eliminated at 226Hz in the preterm infant. Patterns (226 and 300Hz) are generally more complex than the term infants, with a greater prevalence of 5B and 3G patterns. At 400Hz, there are no classified (100% 'Other') preterm tympanograms.
- There would not appear to be a major interaction of ear canal wall distention with either term or preterm infants. The occurrence of the 0B1G Vanhuyse pattern is very low.
- The neonate ear canal does not behave as an acoustically rigid structure. Extreme pressure tail values show high conductance suggestive of energy absorption. It is proposed that this is due to the nature of the ear canal tissues. It is suspected that this is linked with the oedematous conditions of ear canal tissues.
- The majority of tympanometric data display a high level of asymmetry. This behaviour is observed with notch shoulder morphology and extreme pressure tail values. This characteristic is more pronounced at the higher frequencies. It is

proposed that this is related more to the vibratory motion of the ear canal tissues than to wall distention.

- The presence of a mild negative pressure bias for static peak location is confirmed. Peak locations at higher pressures were not encountered. Preterm data does not show any deviation from this behaviour.
- Resonant characteristics for the term infants indicate that 50% display a resonant frequency <226Hz, 39% have resonance between 226 and 300Hz, with 4% displaying resonance between 300 and 400Hz. Seven percent show no resonant characteristics before 1000Hz. All preterm infants show resonant characteristics <226Hz throughout the preterm period.
- Beyond 20 days after birth, all preterm infants display mass controlled susceptance at 226Hz. There are no clear maturational trends in the susceptance data for the preterm infants at 678 and 1000Hz.
- Normative data for both term and preterm (all data collected from the preterm group) populations was established for admittance, susceptance and conductance over the frequency range of 226 to 2000Hz.
- The 226Hz probe tone is not appropriate for the testing of preterm infants during the term period. The majority of infants are still mass controlled resulting in poor tympanometric sensitivity. In comparison, 50% of term infants are mass controlled at 226Hz. At 678Hz, 96% of term infants, and four (of the seven) preterm infants are stiffness controlled. This frequency is recommended for optimum sensitivity. An appreciation of the characteristics of neonate data is required for interpretation. All preterm infants (79% of term infants) are stiffness controlled at 1000Hz. This frequency is not recommended due to the unusual morphology and the increased violation of theoretical assumptions.
- Impedance component data trends tend to be more variable than the admittance data. The reactance-resistance (X-R) relationship is useful in assessing notching in admittance component tympanograms. The reversal of the X-R relationship (from $R>X_s$ to $X_s>R$), reported for term infants, is not observed with the preterm data. This indicates that there is a difference in the development of ME status between term and preterm infants.

Table 6.1 Percentage Vanhuyse classified admittance (Y) tympanograms (term group, n=28) for Successive runs (data for first run, and after 8 and 28 runs)

Vanhuyse class.	Y226Hz		
	1 run (%)	8 runs (%)	28 runs (%)
1Y	50	32	29
3Y	50	68	71

Table 6.2a Percentage Vanhuyse classified for Successive runs - Term infants tested within 3 days of birth (n=14)

Vanhuyse class.	Y226Hz		
	1 run (%)	8 runs (%)	28 runs (%)
1Y	54	38	50
3Y	46	62	50

Table 6.2b Percentage Vanhuyse classified for Successive runs - Term infants tested after 3 days of birth (within 6 days) (n=14)

Vanhuyse class.	Y226Hz		
	1 run (%)	8 runs (%)	28 runs (%)
1Y	47	27	8
3Y	53	73	92

Table 6.3 Percentage Vanhuyse classified tympanograms for the term group (n=28)

	226Hz	300Hz	400Hz	500Hz	600Hz	678Hz
1B1G	7	0	0	4	7	14
3B1G	43	14	4	0	0	4
3B3G	43	68	36	4	7	0
5B3G	4	11	29	11	0	0
0B1G	4	0	0	0	0	0
OTHER	0	7	32	82	86	82

Table 6.4a Percentage Vanhuyse classified tympanograms for term infants tested within 3 days of birth (n=14)

	226Hz	300Hz	400Hz	500Hz	600Hz	678Hz
1B1G	8	0	0	8	8	15
3B1G	46	15	0	0	0	0
3B3G	31	69	38	0	8	0
5B3G	8	8	31	15	0	0
0B1G	8	0	0	0	0	0
OTHER	0	8	31	77	85	85

Table 6.4b Percentage Vanhuyse classified tympanograms for term infants tested after 3 days of birth (within 6 days) (n=14)

	226Hz	300Hz	400Hz	500Hz	600Hz	678Hz
1B1G	7	0	0	0	7	13
3B1G	40	13	7	0	0	7
3B3G	53	67	33	7	7	0
5B3G	0	13	27	7	0	0
0B1G	0	0	0	0	0	0
OTHER	0	7	33	87	87	80

Table 6.5a Vanhuyse classified tympanograms for the preterm group at 226Hz

Vanhuyse class.	Susceptance/Conductance 226Hz					
	28-30PCA	30-32PCA	32-34PCA	34-36PCA	36-38PCA	38-40PCA
1B1G	0	0	0	0	0	0
3B1G	1	0	0	1	1	0
3B3G	1	2	2	2	3	2
5B3G	1	1	0	1	1	0
0B1G	0	0	0	0	0	0
OTHER	0	1	0	1	0	1

Table 6.5b Vanhuyse classified tympanograms for the preterm group at 300Hz

Vanhuyse class.	Susceptance/Conductance 300Hz					
	28-30PCA	30-32PCA	32-34PCA	34-36PCA	36-38PCA	38-40PCA
1B1G	0	0	0	0	0	0
3B1G	0	0	0	0	0	0
3B3G	1	1	1	2	2	1
5B3G	2	1	1	2	3	2
0B1G	0	0	0	0	0	0
OTHER	0	2	0	1	0	0

Table 6.5c Vanhuyse classified tympanograms for the preterm group at 400Hz

Vanhuyse class.	Susceptance/Conductance 400Hz					
	28-30PCA	30-32PCA	32-34PCA	34-36PCA	36-38PCA	38-40PCA
1B1G	0	0	0	0	0	0
3B1G	0	0	0	0	0	0
3B3G	0	0	1	1	1	0
5B3G	0	0	0	2	2	1
0B1G	0	0	0	0	0	0
OTHER	3	4	1	2	2	2

Table 6.6 Numerical data from Figure 6.4 showing the characteristics of cerumen on the canal wall (pressure range -200 to +200daPa)

Presence or absence of cerumen	Susceptance (B)				Conductance (G)			
	Tail values (mmho)		Static Peak (mmho)		Tail values (mmho)		Static Peak (mmho)	
	-ve	+ve	uncomp.	-ve comp.	-ve	+ve	uncomp.	-ve comp.
220Hz present	1.40	1.40	2.25	0.85	0.00	0.00	0.30	0.30
220Hz absent	1.10	1.10	2.25	1.15	0.00	0.00	0.30	0.30
660Hz present	3.50	4.60	3.70	0.20	2.20	2.00	9.70	7.50
660Hz absent	3.00	3.30	7.80	4.80	0.80	0.60	5.60	4.80

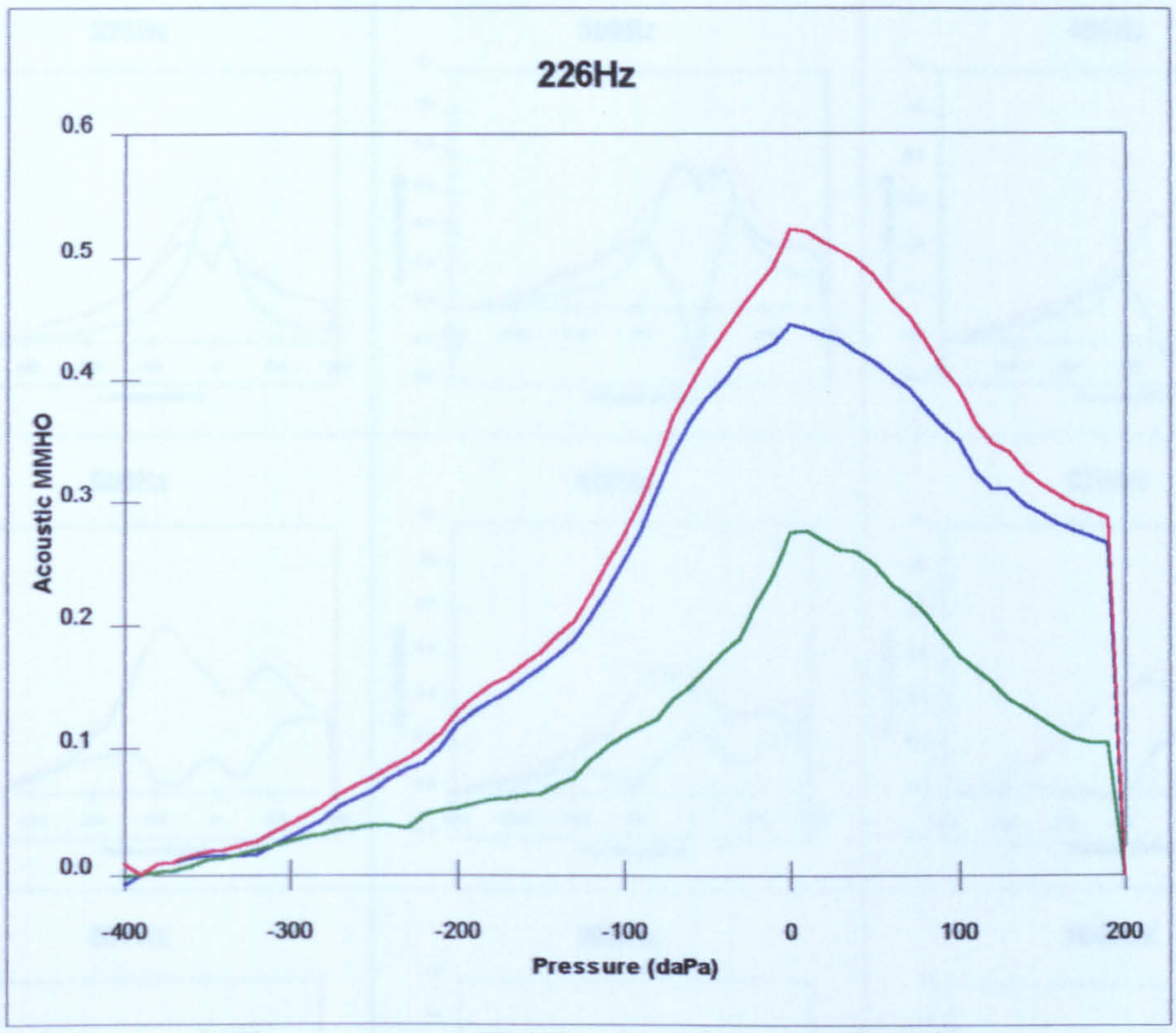
Table 6.7 Percentage of positive and negative static peak susceptance for the term group (compensated to negative tail) (n=28)

Static peak susceptance	FREQUENCY (Hz)												
	226	300	400	500	600	678	800	900	1000	1250	1500	1750	2000
Positive B (%)	50	18	54	89	93	96	96	86	79	50	29	18	11
Negative B (%)	50	82	46	11	7	4	4	14	21	50	71	82	89

Table 6.8 Birth and test information for the Preterm group

Subject	Test session	Gestational age (GA)	Test PCA	Days after birth
DERO	1	28^{+5}	29^{+5}	7
-	2	-	31^{+3}	19
-	3	-	33^{+6}	29
HARA	1	28^{+4}	29^{+5}	8
-	2	-	32^{+0}	24
-	3	-	34^{+5}	43
-	4	-	36^{+5}	57
HARB	1	28^{+4}	29^{+5}	8
-	2	-	31^{+6}	23
-	3	-	34^{+5}	43
-	4	-	36^{+5}	57
JARA	1	31^{+6}	34^{+6}	21
-	2	-	37^{+0}	36
-	3	-	39^{+1}	51
JARB	1	31^{+6}	35^{+1}	23
-	2	-	37^{+1}	37
-	3	-	39^{+0}	50
LOBO	1	36^{+3}	37^{+2}	6
-	2	-	38^{+3}	14
NAIL	1	32^{+4}	33^{+4}	7
-	2	-	35^{+5}	22
-	3	-	36^{+6}	30
RIMI	1	29^{+5}	30^{+5}	7
-	2	-	39^{+3}	68

Figure 6.1 Compensated admittance, susceptance and conductance tympanograms recorded from a term infant (subject LAM)



Admittance - RED, susceptance - BLUE and conductance - GREEN.

Figure 6.2 Compensated susceptance tympanogram (3B) recorded from a term infant (subject FOX)

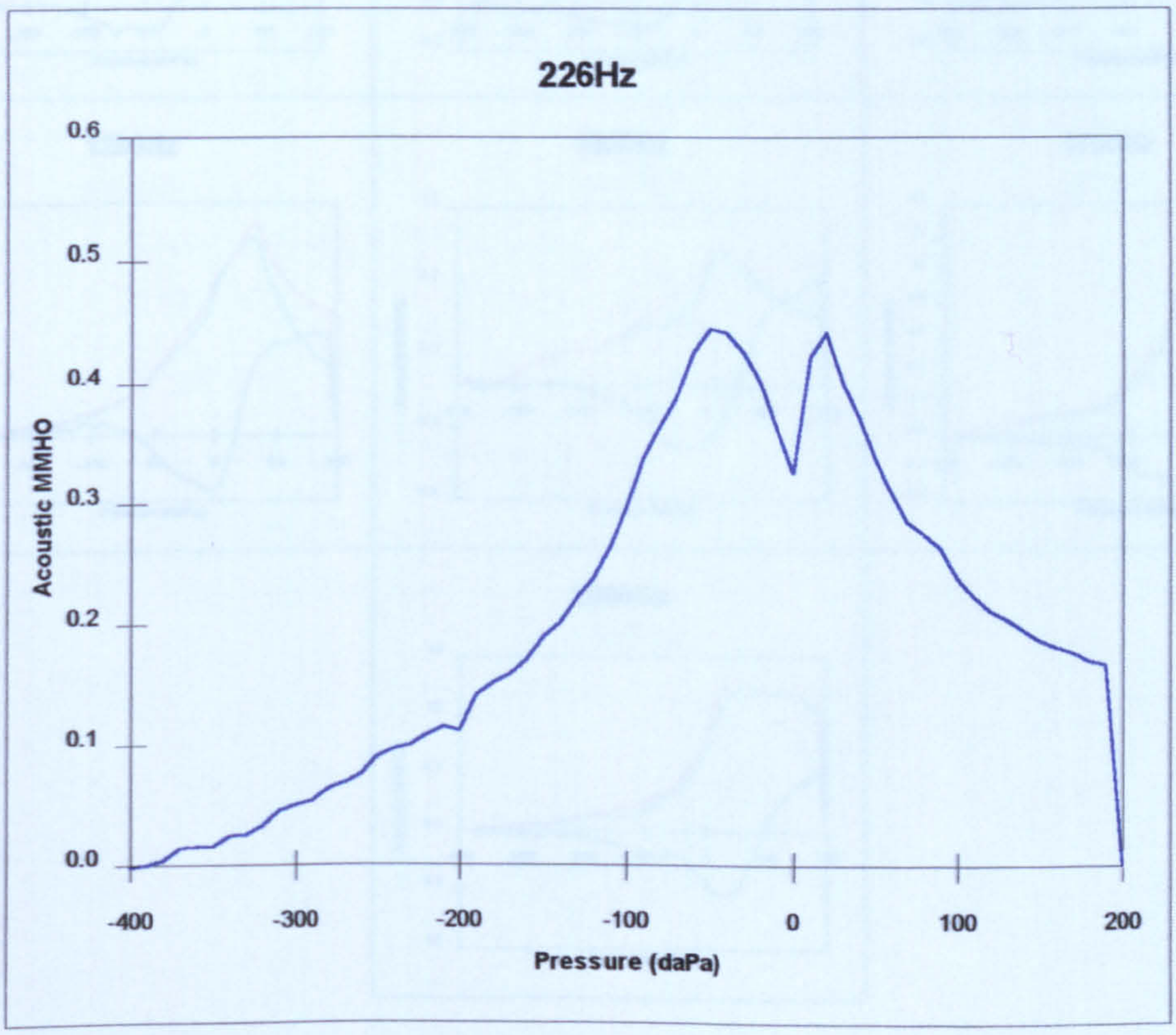


Figure 6.3 Compensated susceptance, conductance and admittance tympanograms for a term infant (subject FOX). Tympanograms are classified as Vanhuyse types 3B1G (226Hz), 3B3G (300Hz) and 5B3G (400Hz).

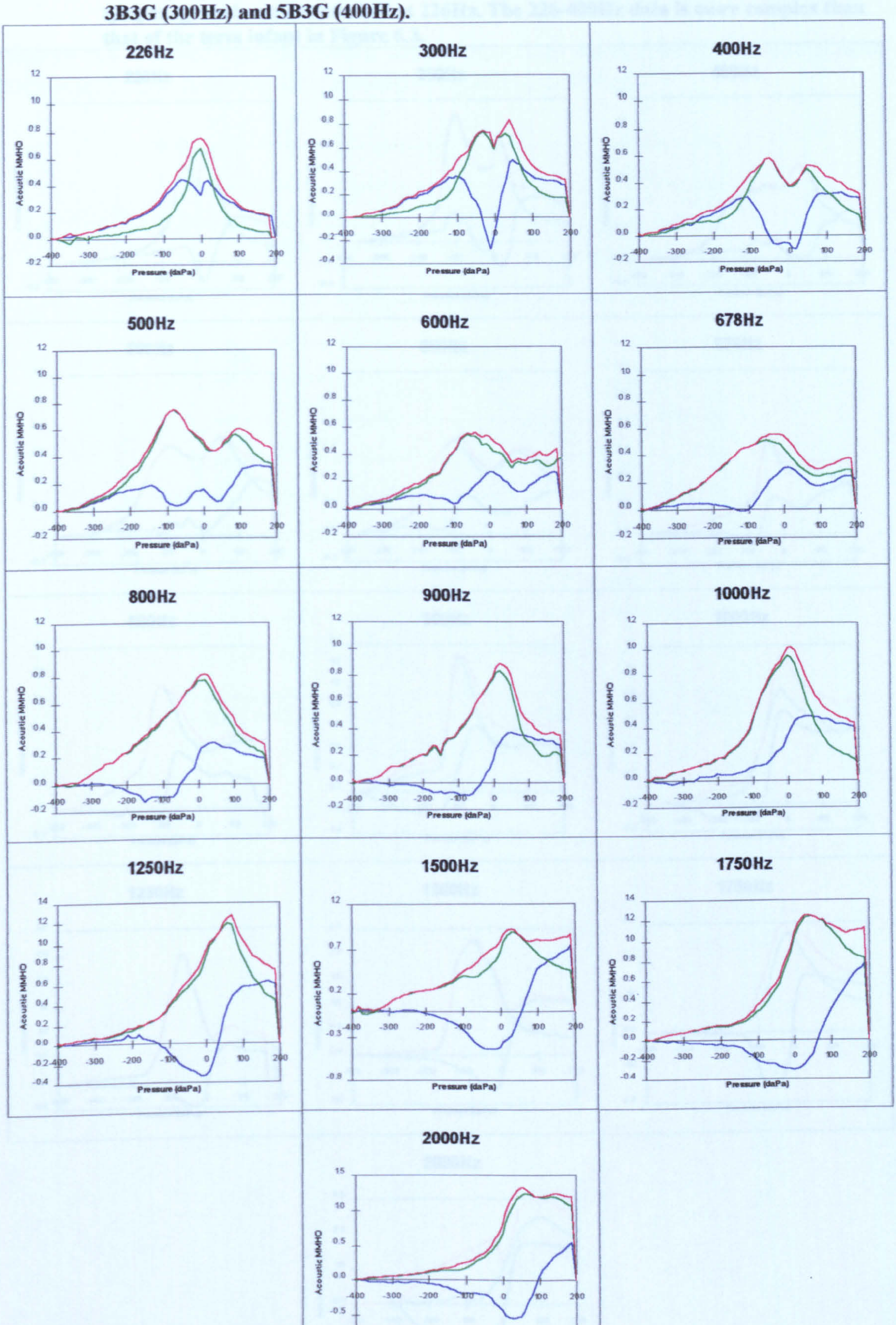


Figure 6.4 Compensated susceptance, conductance and admittance tympanograms for a preterm infant (subject HARA, GA 28^{+4}, tested at 32^{+0} PCA). Note that the susceptance tympanogram is mass controlled at 226Hz. The 226-400Hz data is more complex than that of the term infant in Figure 6.3.

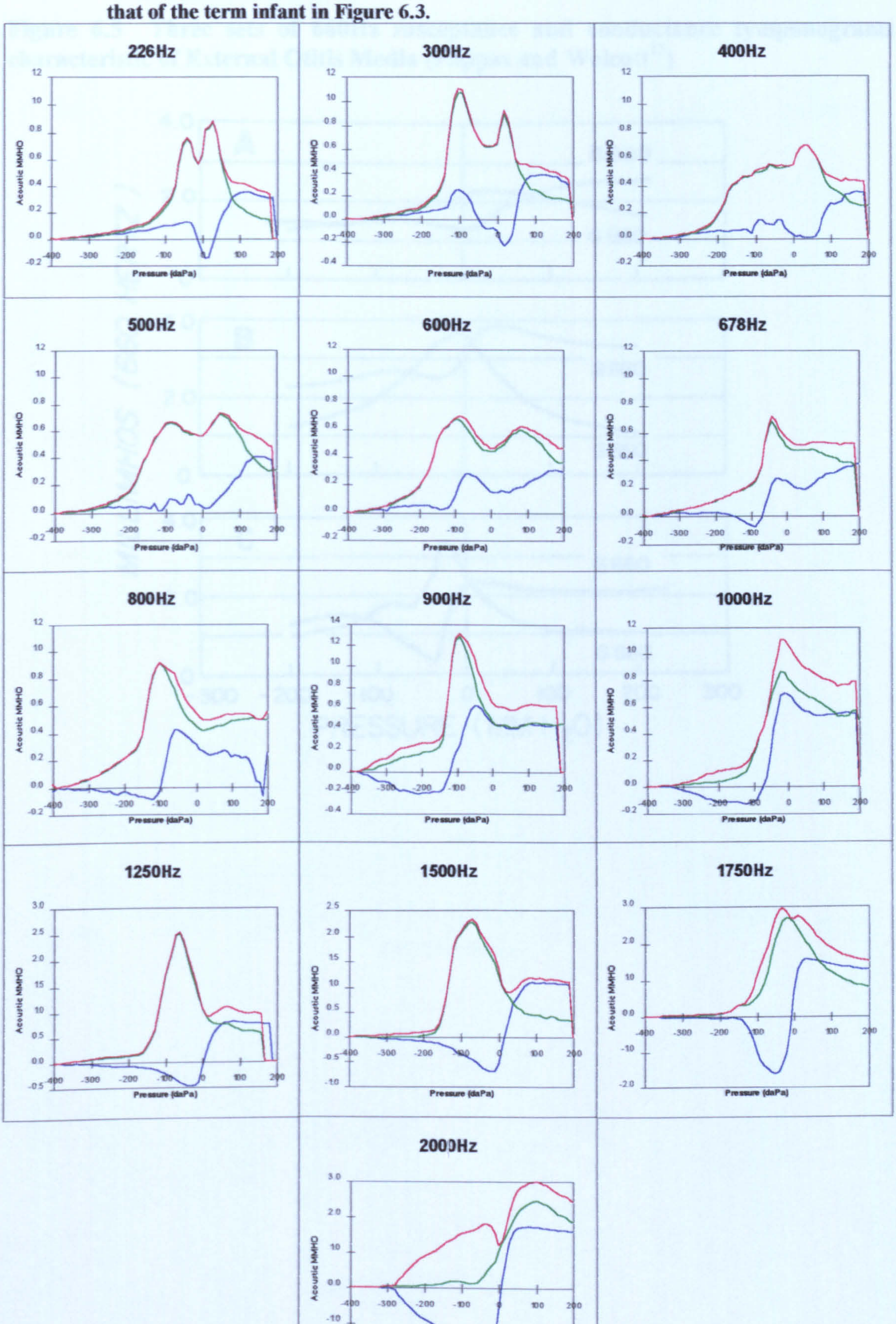


Figure 6.5 Three sets of 660Hz susceptance and conductance tympanograms characteristic of External Otitis Media (Pappas and Wolcott[17])

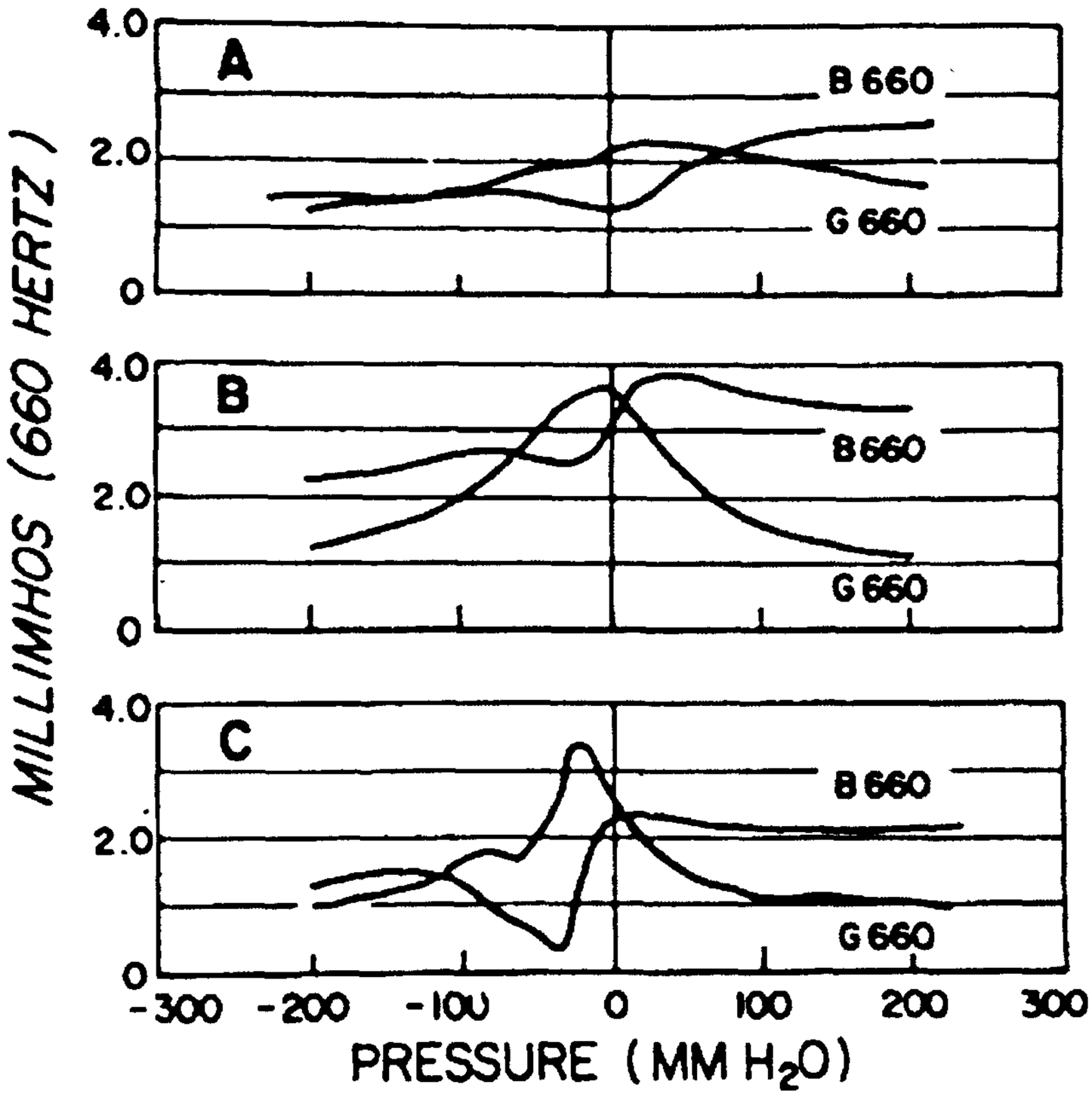
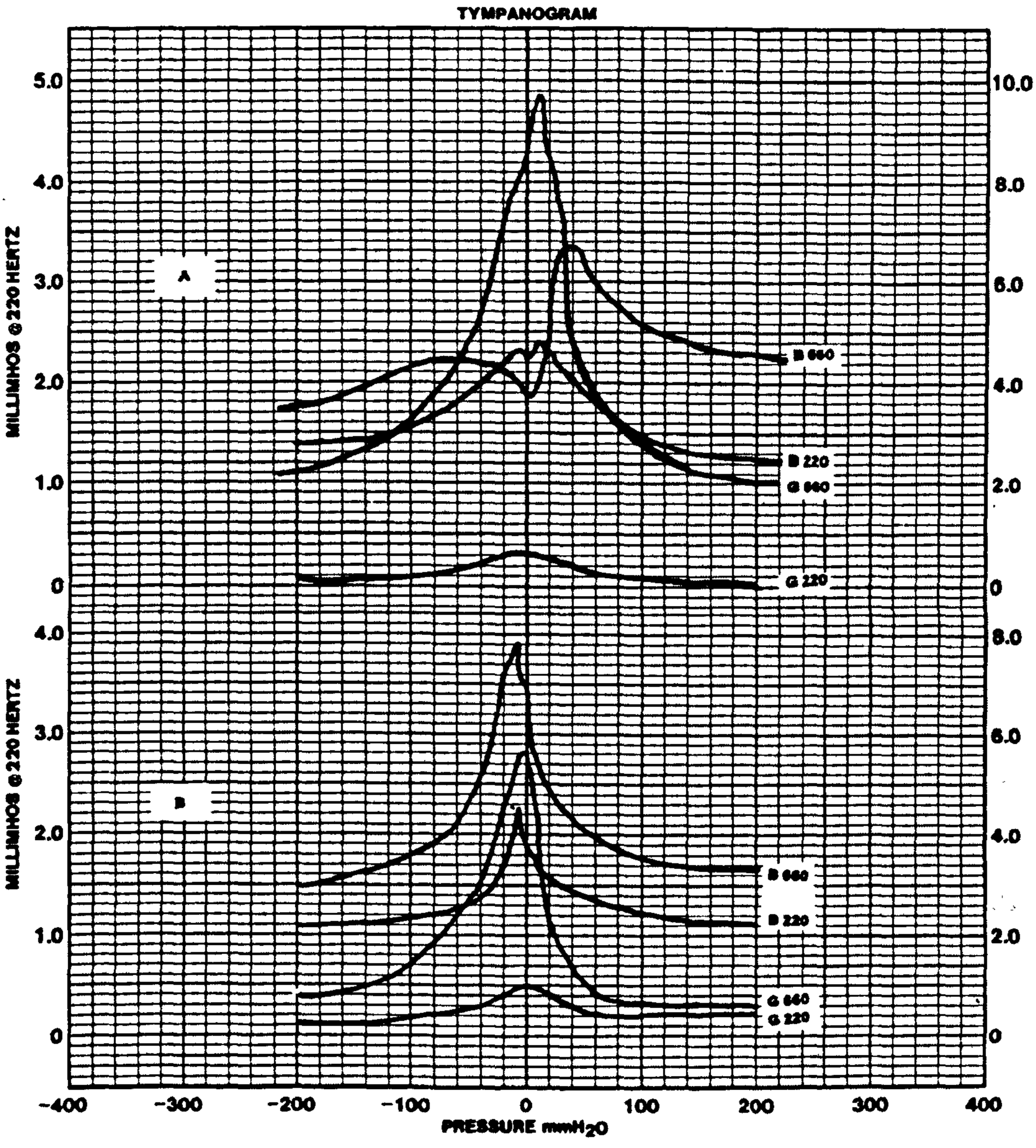
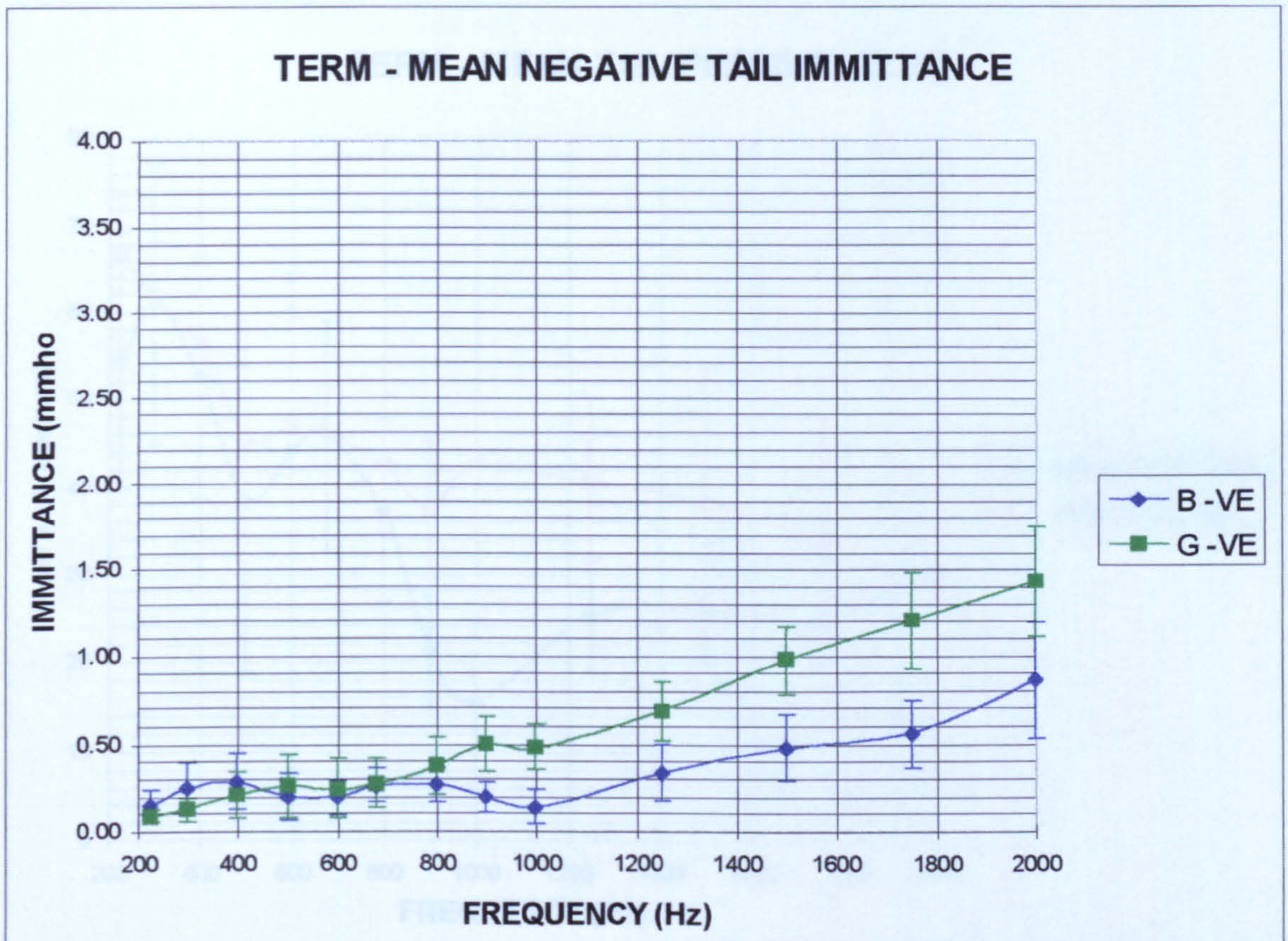


Figure 6.6 Two sets of susceptance and conductance tympanograms (220 and 660Hz) showing the characteristics before (A) and after (B) cerumen removal (Feldman[18])



Figures 6.7/6.8 Mean (S.D.) Negative and Positive tail susceptance and conductance for term group (n=28)



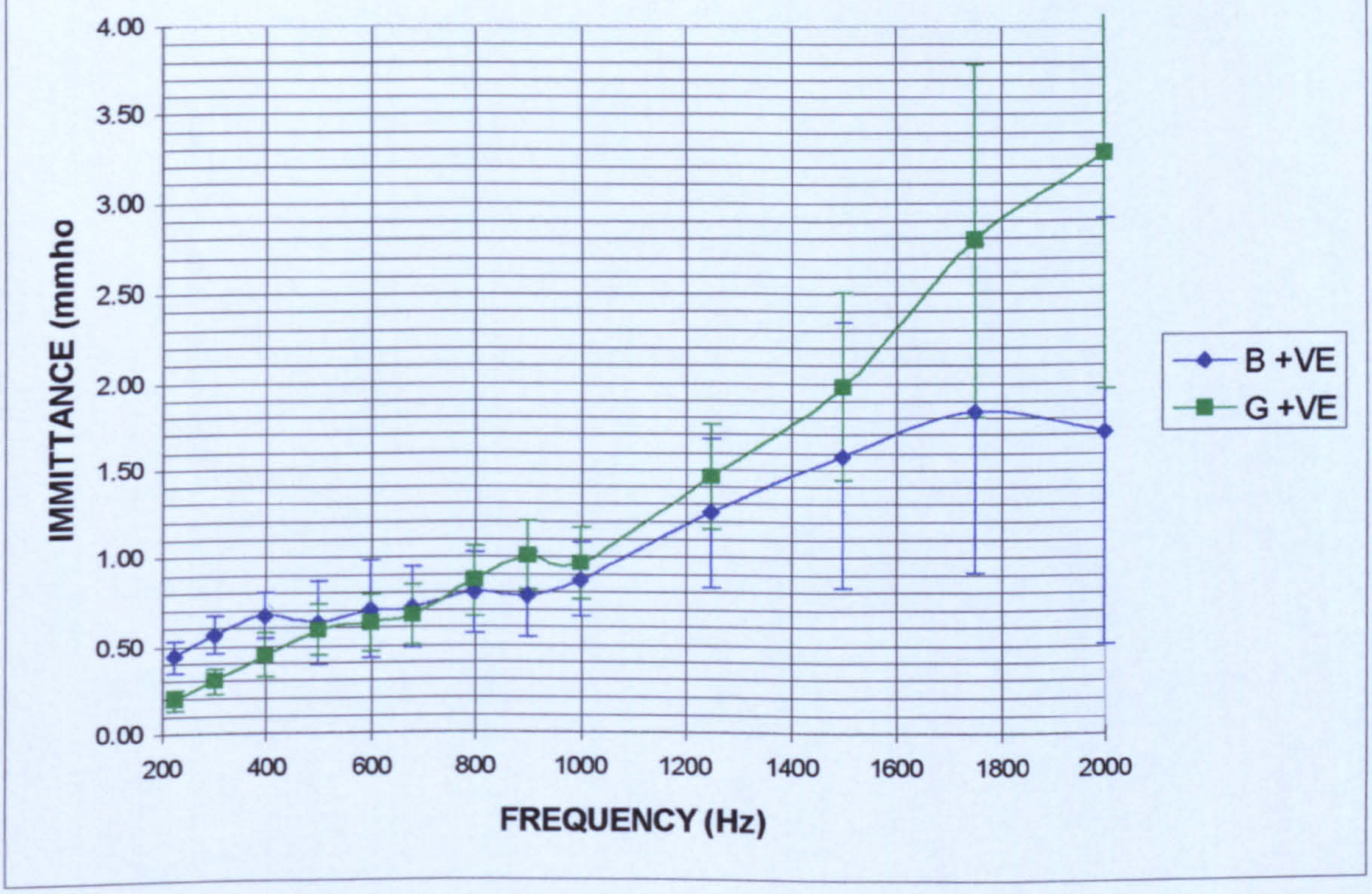


Figure 6.9 Mean (S.D.) Negative and Positive tail phase angles for term group (n=28)

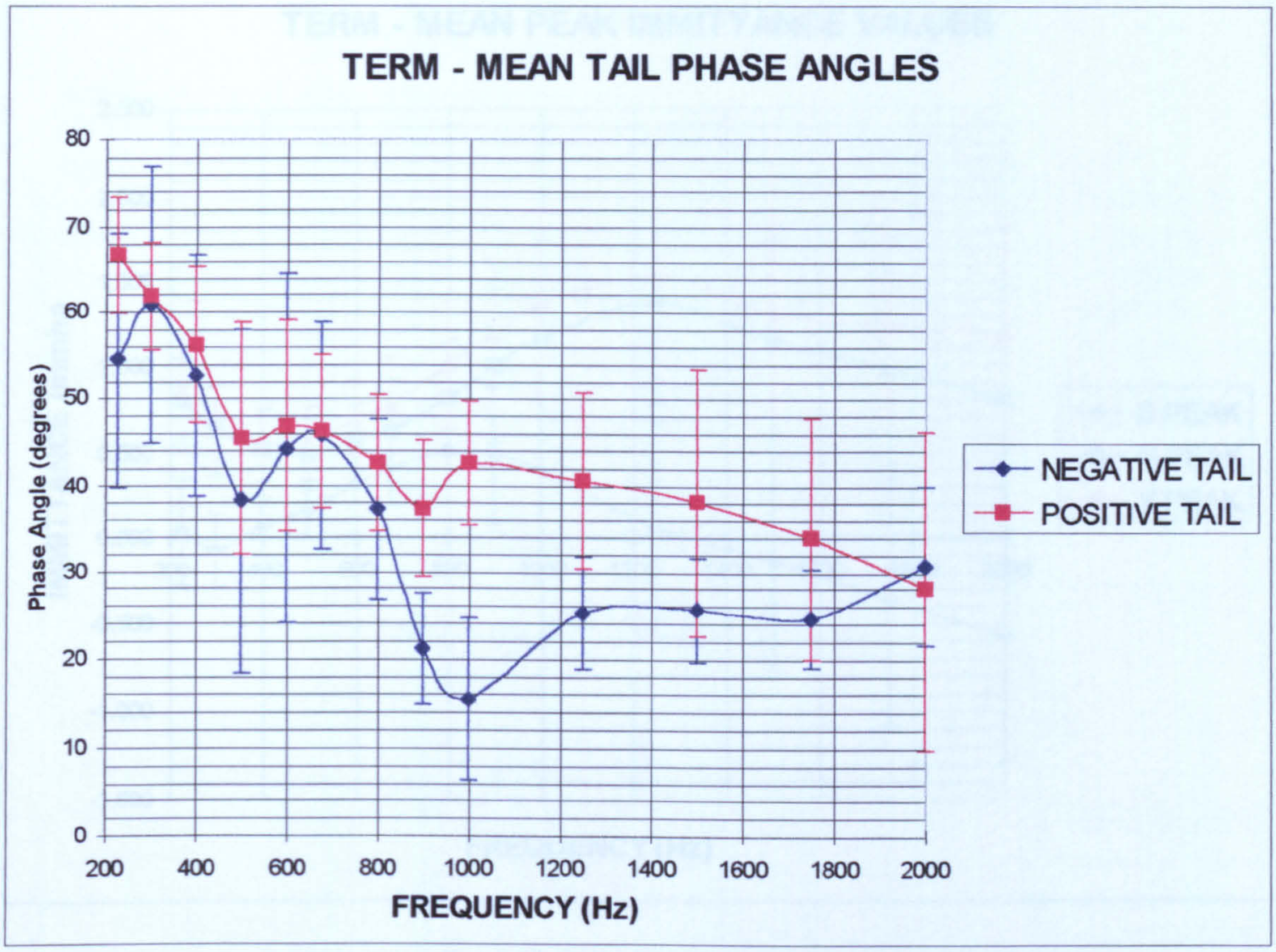


Figure 6.10 Mean (S.D.) static peak admittance component data for term group (compensated to negative tail -400daPa) (n=28)

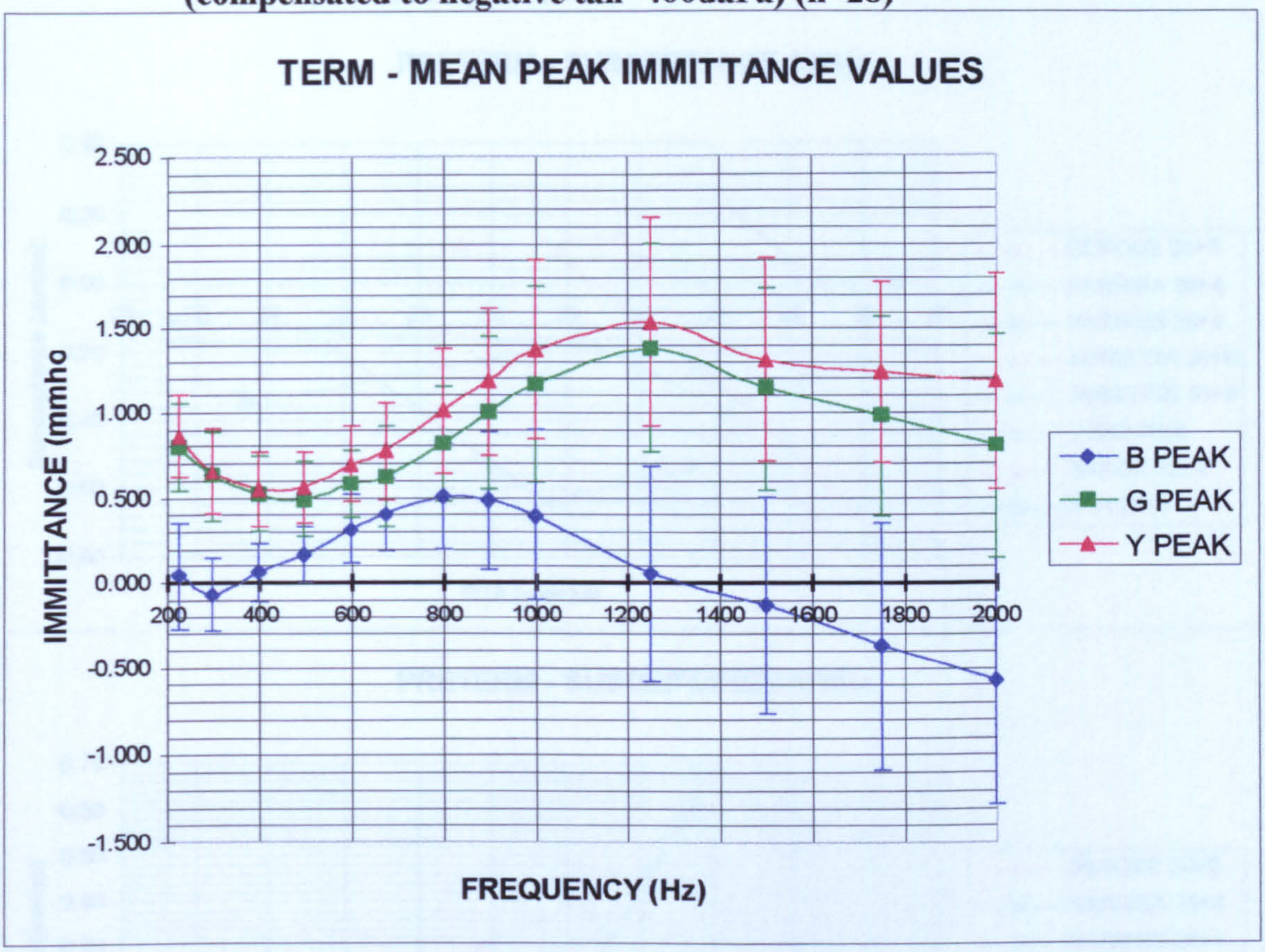
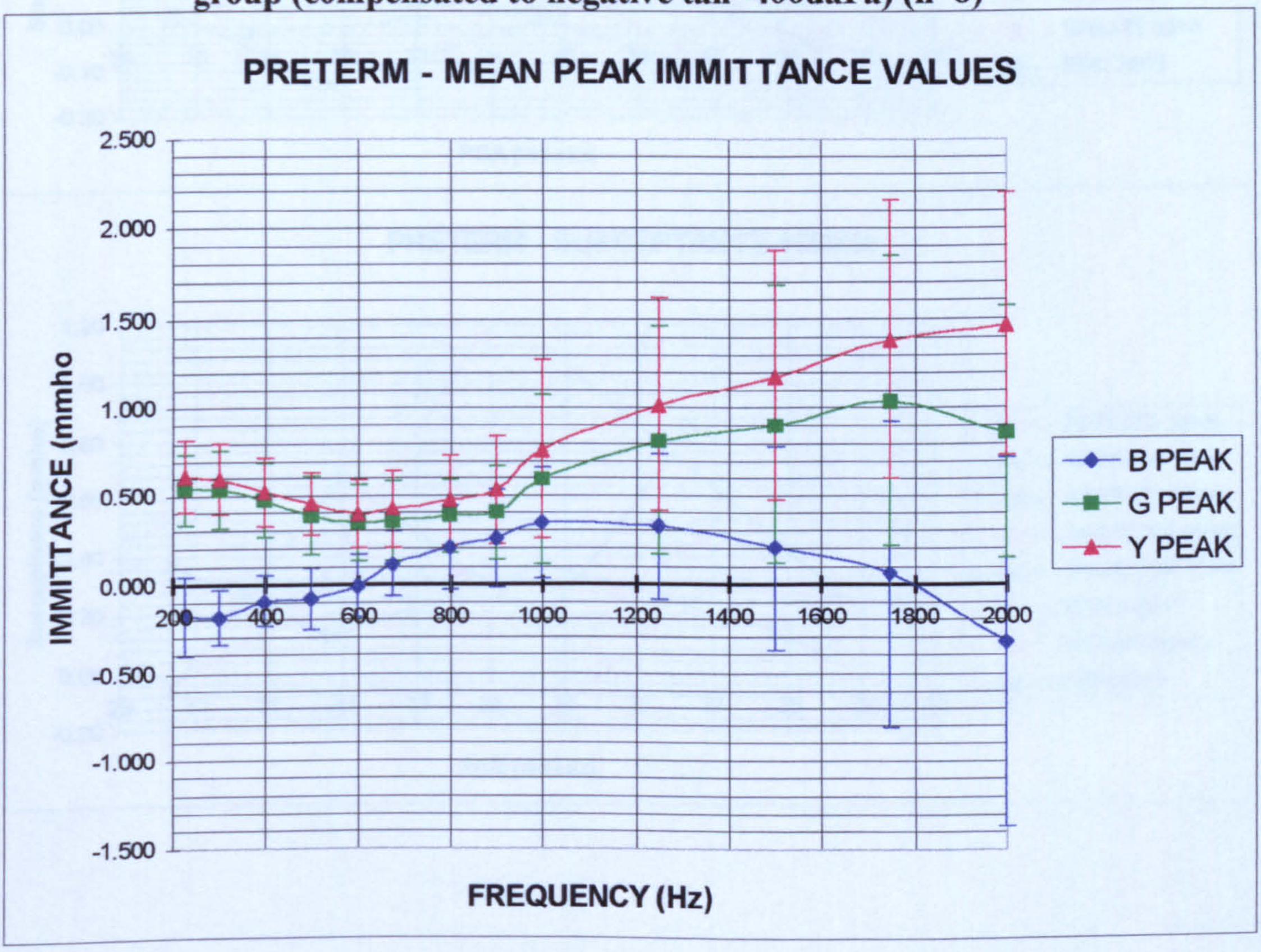
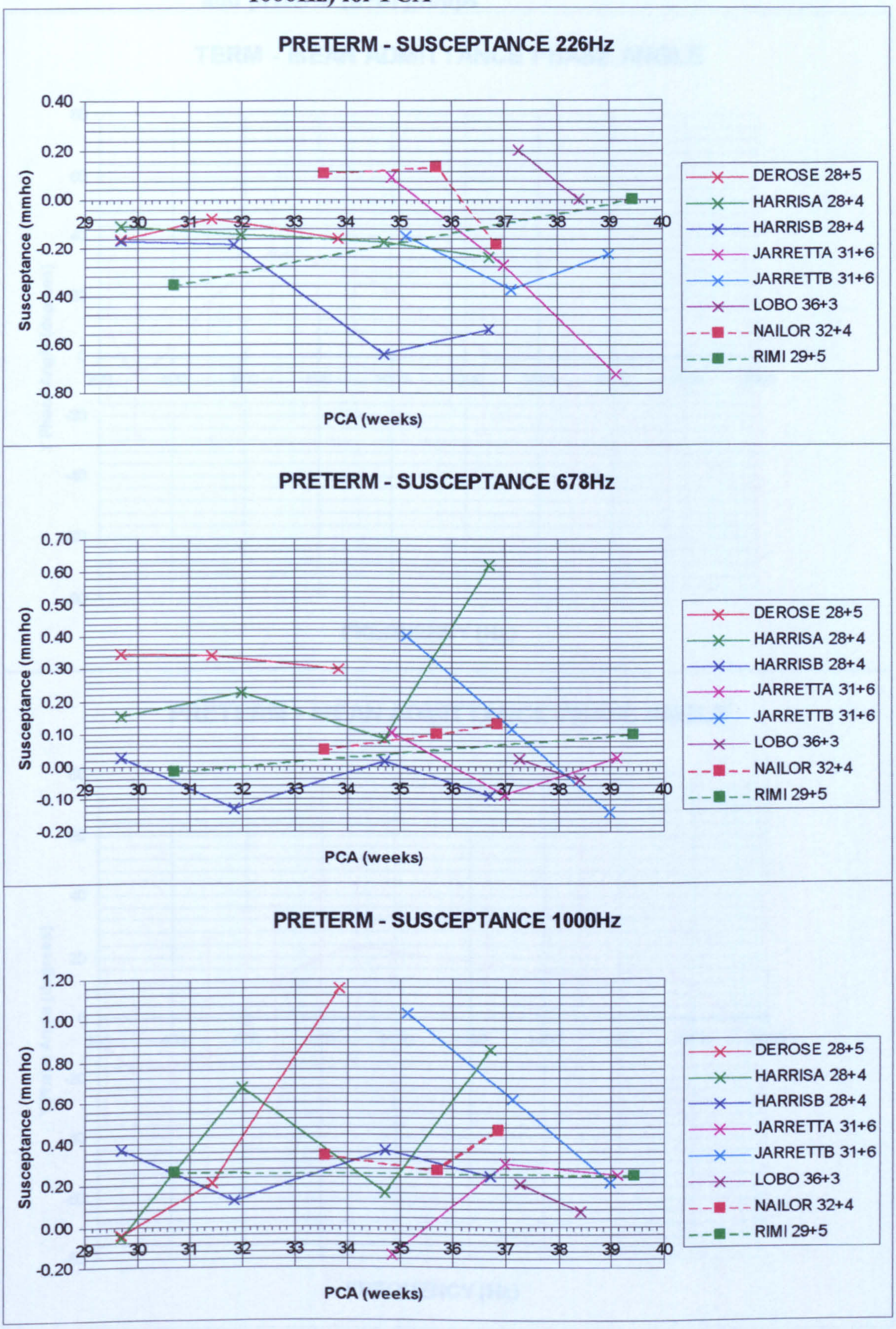


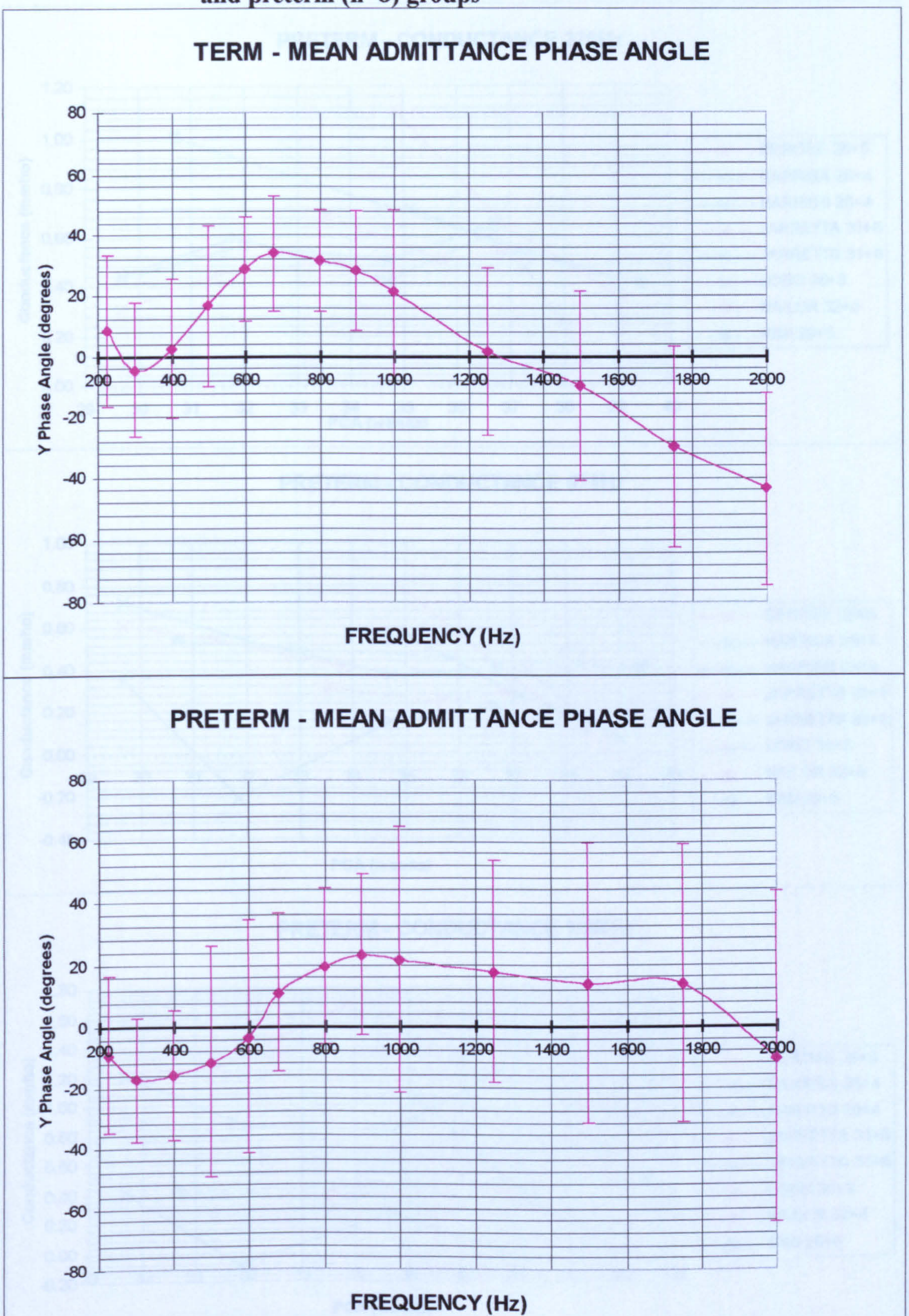
Figure 6.11 Mean (S.D.) static peak admittance component data for preterm group (compensated to negative tail -400daPa) (n=8)



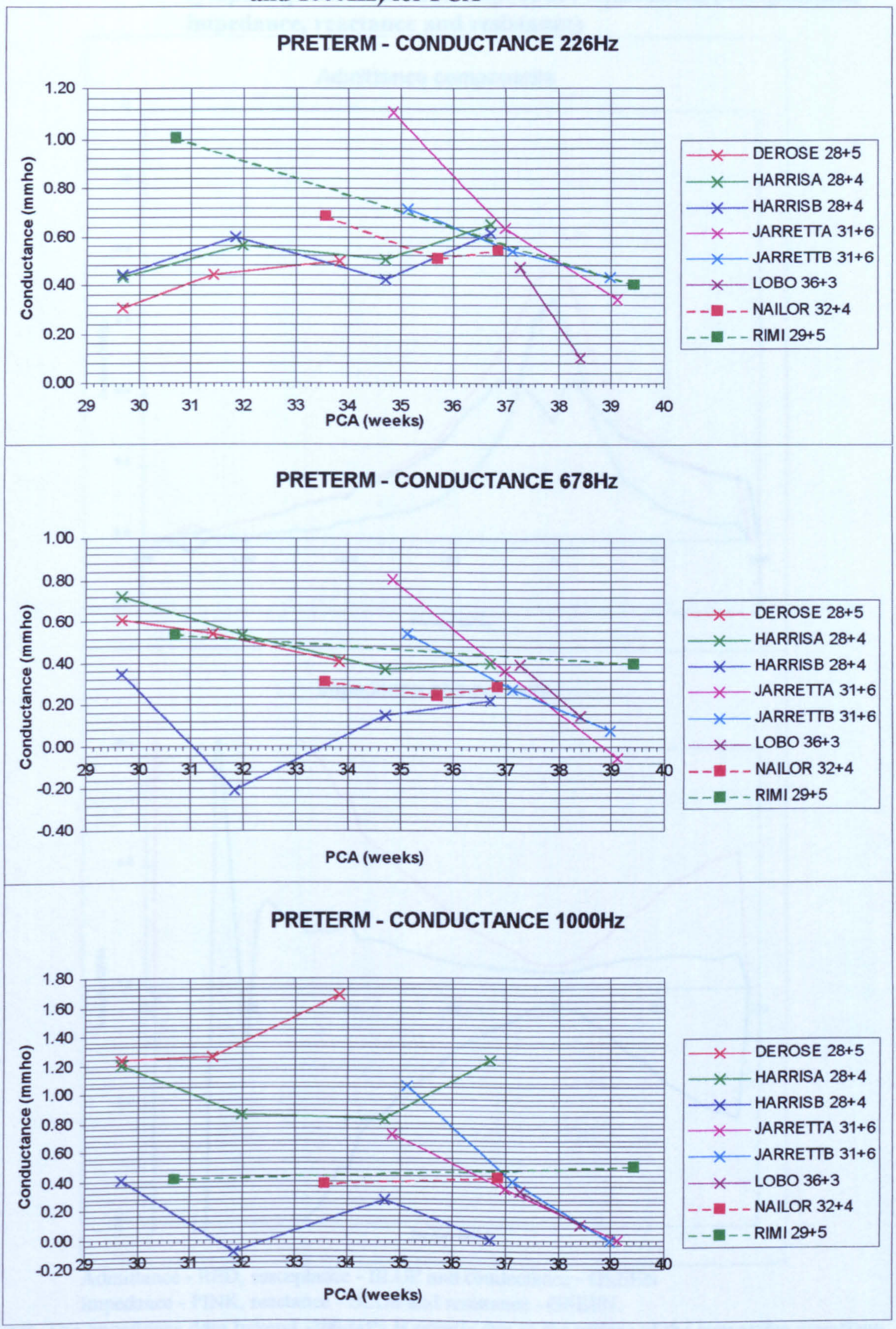
Figures 6.12/6.13/6.14 Preterm static peak compensated susceptance (226, 678 and 1000Hz) for PCA



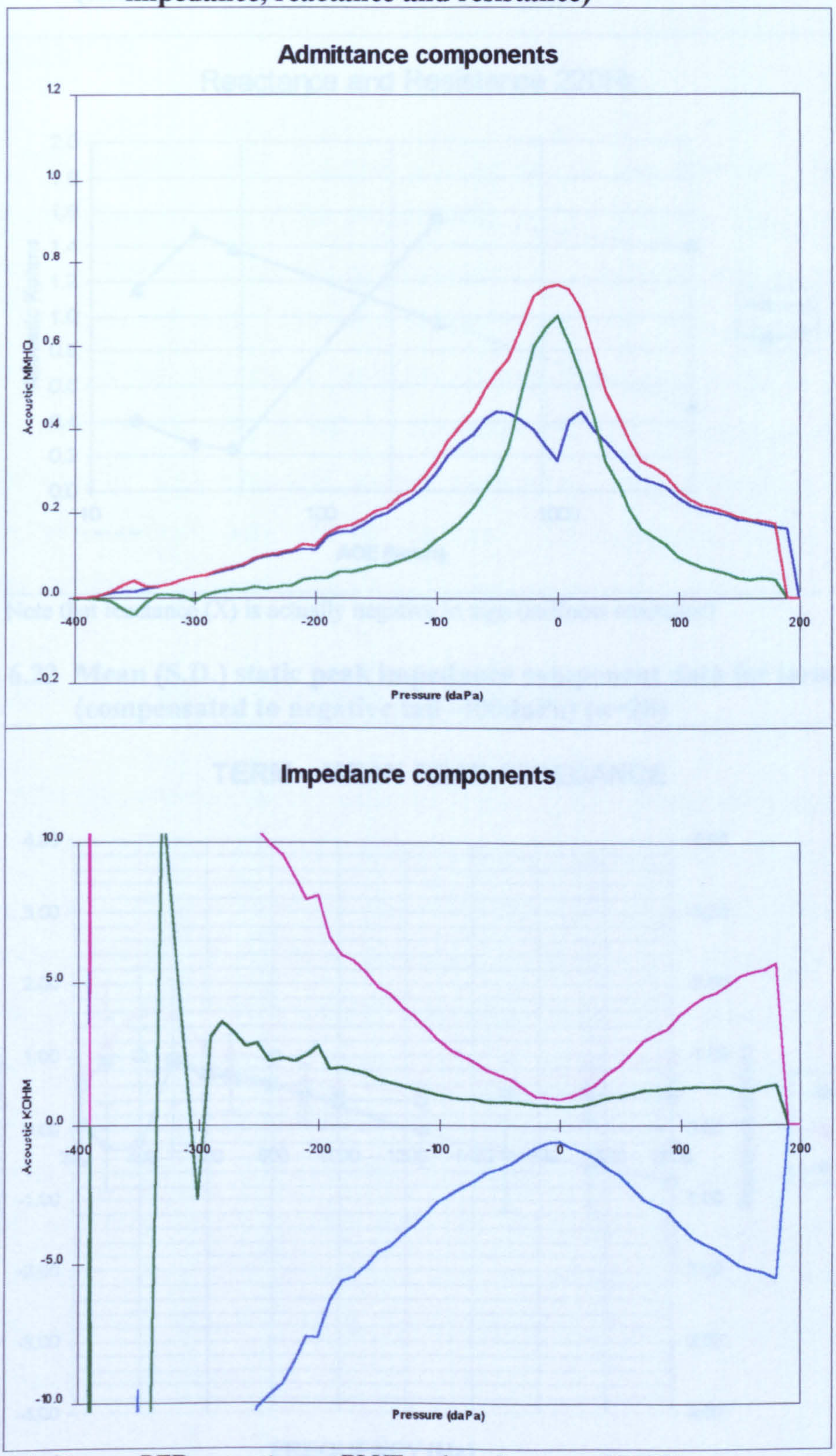
Figures 6.15/6.16 Mean (S.D.) static peak admittance phase angles for term (n=28) and preterm (n=8) groups



Figures 6.17/6.18/6.19 Preterm static peak compensated conductance (226, 678 and 1000Hz) for PCA



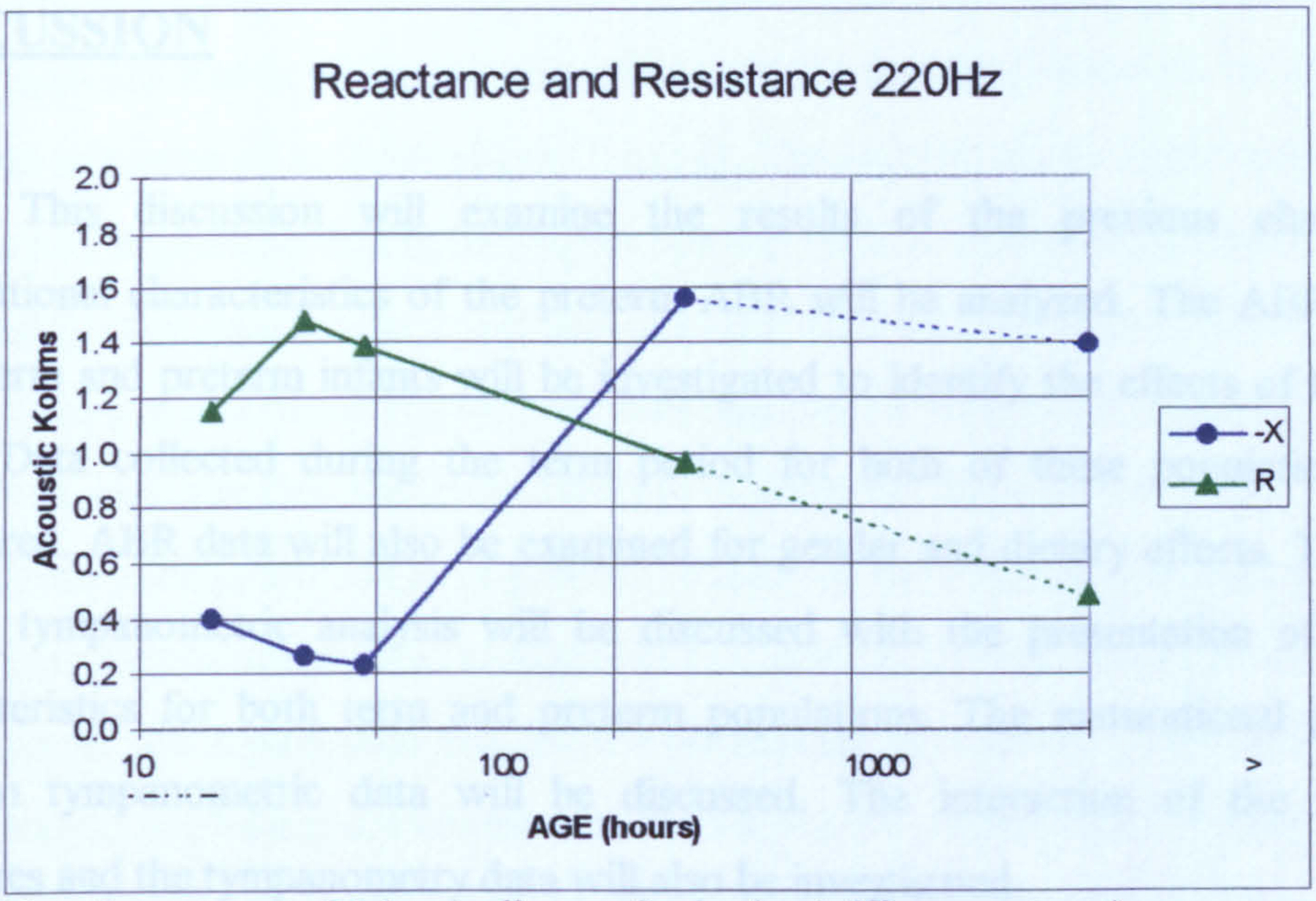
Figures 6.20a/b Compensated admittance, susceptance and conductance tympanograms with their impedance equivalents (compensated impedance, reactance and resistance)



Admittance - RED, susceptance - BLUE and conductance - GREEN
Impedance - PINK, reactance - BLUE and resistance - GREEN.

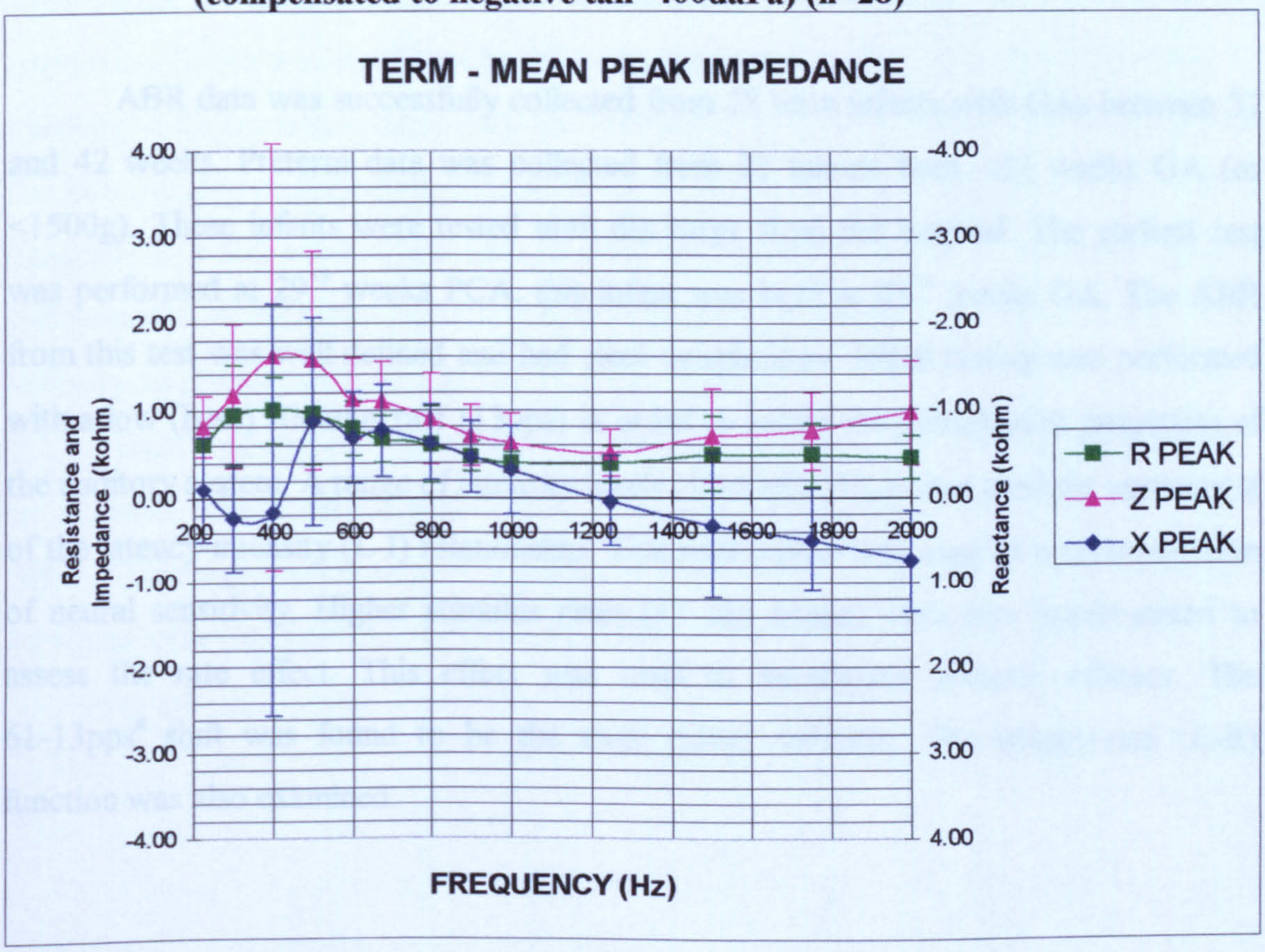
NB. The impedance data beyond -300daPa is erratic due to the nature of the conversion equations in Appendix B. This is not abnormal.

Figure 6.21 Mean values of static resistance (R) and reactance (X) at 220Hz for neonates (first three data points), 2-4 month old infants and adults (Himelfarb *et al.*[25])



Note that reactance (X) is actually negative in sign (stiffness reactance)

Figure 6.22 Mean (S.D.) static peak impedance component data for term group (compensated to negative tail -400daPa) (n=28)



CHAPTER SEVEN

DISCUSSION

This discussion will examine the results of the previous chapters. The maturational characteristics of the preterm ABR will be analyzed. The ABR results of both term and preterm infants will be investigated to identify the effects of the preterm birth. Data collected during the term period for both of these populations will be compared. ABR data will also be examined for gender and dietary effects. The findings of the tympanometric analysis will be discussed with the presentation of normative characteristics for both term and preterm populations. The maturational process for preterm tympanometric data will be discussed. The interaction of the PAS ABR measures and the tympanometry data will also be investigated.

7.1 Discussion of Auditory Brainstem Response (ABR) Results

ABR data was successfully collected from 28 term infants with GAs between 37 and 42 weeks. Preterm data was collected from 22 infants born <32 weeks GA (or <1500g). These infants were tested until discharge from the hospital. The earliest test was performed at 29^{+3} weeks PCA, this infant was born at 28^{+0} weeks GA. The ABR from this test was well defined and had clear morphology. Initial testing was performed with a low (base) stimulus rate (13pps) in order to assess the transmission properties of the auditory system. A range of intensity levels (10-80dB nHL) were used for assessment of the latency-intensity (L-I) relationship. This relationship was used as a representation of neural sensitivity. Higher stimulus rates (37 and 61pps) were also implemented to assess the rate effect. This effect was used to investigate synaptic efficacy. The 61-13pps[a] shift was found to be the most robust measure. The latency-rate (L-R) function was also examined.

[a] 61-13pps refers to the shift in raw latency data between stimulus rates of 13 and 61pps.

7.1.1 Statistical Analysis

The linear regression model was implemented to produce L-A functions to assess the maturational trends of the preterm infants and the variation with GA for term infants. This data is presented in Appendices C and D. The validity of the linear model is tested with the null hypothesis that in the population the slope is zero and that there is no linear relationship. The one-sample t test was implemented with the null hypothesis that the linear regression line is not significantly different from a zero slope. Significance for this test would indicate that the L-A function is different from a zero slope and that a maturational change with PCA is present. Values of the Pearson correlation coefficient (r^2) are also shown on the plots in Appendices C and D. The test for a significant slope is equivalent to the significance test for the correlation coefficient. Confidence bands for the regression coefficient are constructed for all plots in Appendices C and D at the 95% level. These bands display the reliability of the linear regression estimate.

The unpaired t test was used to assess whether two linear regression lines have significantly different regression coefficients. In analyzing whether particular linear regression slopes are significantly different, an assessment of the possibility that the observed differences are due to random variation is required. The null hypothesis is that the two slopes are the same. This analysis was used to assess possible differences in grouped data for gender and diet. It was also implemented to investigate possible differences in maturational characteristics. The maturational rates of different parts of the auditory system were also compared with this method.

Data collected during the term period was tested with the unpaired Wilcoxon test to examine differences between term and preterm data and for gender and dietary effects[b]. The null hypothesis is that there is no tendency for the outcome of one group to be higher or lower than the other. Ranking was corrected for repeated values.

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[b] As stated earlier, the unpaired Wilcoxon and the Mann-Whitney U test are equivalent.

7.1.2 Validity of Data

The variability of preterm ABR data has been well reported[1, 2, 3, 4]. Krumholz *et al.*[1] reported considerable dispersion of all ABR parameters, especially between 28 and 35 weeks PCA. They suggested that this leads to major practical limitations for clinical use in the preterm population. Goldie[3] reported that the concept of standard deviation (S.D.) is of very little use with preterm data. In children and adults, the limit of normality is commonly stipulated as being approximately 3 S.D.s above the mean[5]. In the newborn the variability makes it difficult to use such criteria for defining abnormality.

Issa and Ross[2] attempted to construct a more reliable normative data set for the preterm population. They suggested that the increased S.D.s found in the younger subjects could be due to errors in the estimation of conceptional age. They indicated that these errors would not, however, account for all of the variability. The conception age information used for this current study was based on the values used by the medical staff in the NICU. Observing the raw data from this current study, error in conceptional age estimation could not account for the considerable variability of the data. Maturational trends were often inconsistent, with erratic behaviour being observed. This was found with all ABR parameters, the IPL measures were most affected. Durieux-Smith *et al.*[4] also found much greater variation, with increased S.D.s, for IPL measures than absolute latency data.

7.1.3 Analysis Discussion

7.1.3.1 Latency-age (L-A) Functions

One of the aims of this study was to examine the maturational characteristics of ABR data in the preterm infant. Other factors such as gender and diet were also considered but the emphasis with these factors was on the effects by the term period. A number of different strategies were considered for investigation of the maturational characteristics.

Preliminary examination concentrated on developing maturational indices for individual subject data. The number of data points, and the degree of natural variability in this data, is of concern when attempting to apply regression analysis. Preterm infants

were tested between three and five times during the approximately ten week preterm period under investigation. There is a natural variation in initial test and final test PCA. In addition, some variation also exists in the fortnightly test PCAs during this period. Testing time was obviously governed by the availability of subjects due to medical treatment, parental visits and staff availability. An increased frequency of testing would have enabled a more accurate maturational model to be developed for the individual subject data. However, with test sessions taking 1.5-2 hours to perform, testing at a greater frequency was deemed to be too intrusive to the medical treatment of the subjects. It should also be noted that the use of individual subject data separately relies on the accuracy of PCA estimation which can be subject to considerable errors.

Individual L-A functions of various regression models were constructed for preterm infant data. Eggermont and Salamy[5] suggested double exponential curves to describe maturation over this period. They indicated particular time constants for various ABR parameters. They indicated greater reductions in latency before 32 weeks PCA with lesser trends beyond 32 weeks to term. The data from this current study was predominantly beyond 32 weeks PCA and so the use of double exponential curves was not suitable. Single exponential curves were not found to provide reliable estimates of the underlying maturational characteristics. Linear regression L-A functions also produced data that was highly dependant on test PCA. This provides maturational data that is more indicative of the test PCA characteristics than of the true maturational behaviour. Infants tested soon after birth (at early PCAs) display prolonged latencies upon initial testing, this increases the maturation as denoted by the L-A function regression coefficient. Those infants where initial testing was delayed due to clinical considerations do not show this initial extreme prolongation of latency data. Their L-A functions, thus, suggest a lesser maturation. The initial test PCA was very variable due to the individual medical conditions encountered by the infants. The erratic nature of the maturation process and the varying PCA of the final testing also had a significant affect on individual L-A functions. The inaccuracies in individual L-A functions is further compounded by the variation in the number of test sessions. Some subjects were only tested on three occasions, this provides lesser reliability to the linear regression analysis than subjects tested on four or five occasions. The construction of L-A functions using linear regression analysis on just three data points is unsound and statistical significance

cannot be achieved. Methods of improving the linearity of the maturational characteristics were investigated. A logarithmic transformation was used to compensate for the deviations from linearity, occurring especially at the earlier test PCAs. Considerable variation in maturational indices was still observed.

Matthews *et al.*[6] reported on the benefits of the reduction of data to single descriptive values. They suggested that target values could be used to assess maturational behaviour. Mean term values for the various ABR parameters were implemented as target values for preterm maturation. This method is beneficial in that it gives a direct comparison of particular preterm infant characteristics and the normative characteristics expected by the term period. Again, a large amount of variability was observed in the PCA values for the target data. These values were again significantly dependant on test characteristics rather than maturational behaviour. No useful data was generated.

Normalization of preterm data onto the data acquired from the term infant population was considered. This would be beneficial in allowing direct comparison with the term infants throughout the maturation of the preterm period. Percentage values of preterm data relative to mean term data could be used. However, it is difficult to produce a good reliable method. In order to demonstrate statistical significance for the small differences being examined a larger number of subjects would be required. This would necessitate a large prospective multi-centre trial. The possibility of testing at another centre was investigated but was not found to be practical for this current study. An additional future investigation could be performed with a retrospective Meta analysis which would allow for analysis of a larger population for the particular characteristics of interest.

7.1.3.2 Group Latency-age (L-A) Functions

Regression was also implemented on group data for all preterm infants. This method reduces the influence of variability in subject testing characteristics that distorts maturational characteristics with individual subject data observations. As previously mentioned, the effects of the preterm birth are assessed by direct comparison of data collected from the preterm infants during the term period with normative term data. The

maturational characteristics during the preterm period are chiefly concerned with different rates of maturation between various ABR parameters and at particular stimulus presentation rates. Group analysis is therefore a valid method for this kind of investigation. It also has the benefit of reducing the affect of PCA estimation errors that are more intrusive on individual subject data.

Selection of an appropriate regression model was performed by calculation of the residual variance for linear and polynomial trends for all ABR parameters. The variance ratio can be used as an assessment of the true best regression fit for the data. This was performed for all ABR parameters. It was found that no more reliable estimation of trends in the data could be achieved by the implementation of a quadratic model over a linear model (no statistical advantage could be identified for any of the parameters). The selection of a linear regression model was further assessed using the Runs test. This test investigates whether a regression trend is a true description of the underlying data or whether there is a significant deviation from the implemented model. There was found to be no significant deviation of the data from the chosen model for all ABR parameters.

Significance was achieved for the Pearson correlation coefficient (r^2) for the majority of preterm group data. This shows a good level of reliability for the linear trends implemented. In addition, confidence bands for the regression lines at the 95% significance level were also constructed. These can be seen in the plots of Appendices C and D.

The validity of this method is supported by the correlation of known maturational data for ABR parameters with the regression coefficients observed for the current study. The numerical data for these known relationships correlated well. The reliability of the L-A functions for this study were assessed by comparison of the relationships suggested by absolute latency trends. Generally, IPL data L-A functions behaved in the manner indicated by the absolute latency trends. Numerical data was found to behave well. The behaviour of the group L-A functions was also supported by the data at different stimulus rates. The higher stimulus rate data generally displayed higher gradients indicating a reducing rate effect with time. The convergence of high stimulus rate data to that of a lower rate was confirmed with L-A functions for rate shifts (eg. 61-13pps) calculated from raw data. Numerical data from these L-A functions correlated well with category mean behaviour.

7.2 Hearing Loss Assessment

7.2.1 Preterm Data

In order to study the maturational characteristics of the preterm infants it is necessary to eliminate any cases of hearing loss. It should be noted that all the infants (n=22) passed the standard ABR screening for high risk babies (performed >36 weeks PCA, before discharge from the hospital). The hearing assessment for this study is based on information from latency-intensity (L-I) data and from threshold estimation (wave V). It should be noted that, due to time constraints and test environment, thresholds can be slightly elevated. Infants were tested to a minimum intensity level of 10dB nHL.

Despland and Galambos[7] first described criteria to diagnose audiological and neurological abnormality. They stipulated that, for a normally functioning auditory system, waves I and V and the I-V IPL should be within 'normal' limits, and that responses are evident at 30dB nHL. Audiological abnormalities are generally of two types; conductive or sensorineural (SN)[8]. A conductive hearing loss is suggested by a reduction in auditory sensitivity. This reduction is caused by the peripheral auditory system (PAS). This condition produces an increased ABR threshold with a prolongation of wave I (and thus the absolute latency of wave V) together with a normal I-V IPL. It affects latency-intensity data, moving the function laterally but maintaining its parallel orientation to the normal function. A SN hearing loss is probable if waves I and V, and the I-V IPL are within normal limits at higher intensity levels. The ABR threshold will, however, be elevated with increasing wave V latency at lower intensities. This increases the L-I function gradient.

Neurological function is generally assessed with the I-V IPL at supra-threshold levels (>50dB nHL). This is a measure of the central conduction time[9]. Neuropathology or neuromaturational delay (affecting auditory pathways of the brainstem) is suggested if a normal wave I is accompanied by a prolonged wave V and I-V IPL. Follow-up testing allows for distinction between maturational processes and permanent damage to the brainstem. Poor waveform morphology is also indicative of neurological dysfunction[10].

7.2.1.1 Threshold Estimation

ABR threshold is defined as the lowest intensity level where a distinct wave V can be identified. Five preterm infants (of 22) showed elevated ABR thresholds >30dB nHL (all being 40dB nHL). All of these occurred during initial testing and subsequently reduced to 30dB nHL or less by the next test session. The test PCAs for increased threshold ranged from 29^{+5} to 32^{+4} weeks. All these tests were performed on infants treated in incubators in the Neonatal intensive care unit (NICU) environment. L-I data for four of these infants was indicative of both SN and conductive abnormality. The other infant just displayed a lateral movement of the L-I function indicative of a conductive interaction.

Whilst using a threshold criteria of 30dB nHL for normal auditory sensitivity, a threshold of 40dB nHL for infants of this PCA is not significantly abnormal. Lary *et al.*[11] reported on the maturation of ABR thresholds during the preterm period. They suggested 40dB nHL at 28-34 weeks PCA, 30dB nHL at 35-38 weeks, and <20dB nHL for term infants. Krumholz *et al.*[1] suggested that threshold maturation could be due to factors affecting conduction velocity; such as, degree of myelination, axonal diameter and synaptic efficacy. ABR thresholds identified in this study would suggest lower values than those reported by Lary *et al.*[11]. All infants display thresholds at 30dB nHL or below in the 28-34 week PCA period, 82% having thresholds <30dB nHL. For those preterm infants tested during the term period (>37 weeks PCA), 100% had thresholds at 20dB nHL or below. It is clear, from threshold estimation, that none of the infants on the preterm study were affected by a permanent hearing loss.

7.2.1.2 Latency-Intensity Relationship

Whilst the ABR thresholds for these infants are within the normal range, there is evidence of neural and conductive maturational processes. The mean term L-I exponential was used to assess any gradient increases at lower intensities and lateral movement of the functions. The presence of neural dysfunction or immaturity is displayed by an increase in the L-I gradient at lower intensity levels. Conductive development is shown by a lateral movement parallel to the term exponential (more

clearly shown at supra-threshold levels). The majority of L-I functions approach the term mean function with age. A reduction in elevated latency values at lower intensity levels was also observed. These maturational characteristics will be discussed later in this chapter.

7.2.2 Term Data

The hearing assessment for the term study is based on the same assumptions as those mentioned for the preterm infants. The absolute latency wave V threshold was estimated and the L-I function constructed for all term infants. Considering threshold estimation; 46% of the infants have wave V thresholds at 10dB nHL, 25% 20dB nHL and 25% 30dB nHL (one infant having a threshold of 40dB nHL). With a pass criterion of 30dB nHL, one infant (subject WH1) fails. Neural function can be assessed with the L-I function at low intensity levels. Two infants display increased exponential gradients at lower intensities. These infants are subjects BYE (30dB nHL threshold) and WH1 (40dB nHL threshold).

Subject BYE displays a very high L-I function gradient of 1.266ms/10dB nHL, compared to a term mean of 0.426ms/10dB nHL (n=28 S.D. 0.152). This suggests a level of SN dysfunction. Observing the absolute latencies (60dB nHL) of waves I and V, and the I-V IPL, all these measures are within normal limits. This would suggest that there is no conductive hearing loss and that the central auditory system is not affected by a neuromaturational delay.

Subject WH1 displays a less exaggerated increase in the L-I function gradient (0.870ms/10dB nHL). The lesser value could be influenced by the threshold of 40dB nHL, higher thresholds decreasing L-I gradients when SN characteristics are present. Examining latency measures, both absolute wave I and V display prolonged values. The I-V IPL is within the normal limits. This indicates that, whilst there is no evidence of neuromaturational delay (central system), there is evidence of some conductive PAS dysfunction.

Both of these infants will be excluded from mean calculations and L-A function construction. Data will, however, be included in the scatter plots in Appendix D.

RESULTS ANALYSIS

The term and preterm group ABR results were presented in Chapter Five. The behaviour of ABR parameters was investigated and normative data presented. This discussion of the ABR results will concentrate firstly on the maturation observed in the preterm infants and secondly on the effects of the preterm birth on the auditory system. Preterm mean data for the 36-40 week PCA period (n=17) will be compared with the normative term data (n=28 37-42 weeks PCA). Table 7.1 shows the mean data for term and preterm (term period testing) groups for comparison. Gender and dietary effects will also be investigated.

Maturational Characteristics

7.3 Base Stimulus Rate (13pps)

7.3.1 Intra-uterine Maturation of the Term Infant

By studying ABR parameters of the term infants just after birth it is possible to investigate the variation of the parameters with GA. These relationships can indicate whether maturation of the parameter is still present, or whether it is stationary.

The significance values for the one-sample t test for the L-A functions for the term data can be seen on the scatter plots in Appendix C. These values show whether the L-A functions show a statistically significant gradient compared to a zero slope. At the base stimulus rate, it can be seen that differences in slope from zero rarely exceed the required statistical level of probability ($P<0.05$). This is probably due to the relatively low number of subjects used to construct the L-A functions (n=28) and the errors involved in GA estimation. These characteristics will increase the scatter of data and would thus necessitate large numbers of subjects in order to demonstrate small differences to be significant. This would also be required to minimize type I and II errors. The GAs used for this project are based on those used within the hospital. The error involved with these values was discussed in Section 7.1.2. The base stimulus rate data for the term infants can thus only be used as a general guide of the maturation of the ABR parameters with GA. It is still of interest to undertake this analysis using the same methods as those implemented with the preterm group for comparison.

The wave I latency values show no evolution with GA, values being relatively constant throughout the term period. The overall L-A function gradient is ≈+0.03ms/week. The one-sample t test shows no statistical difference from a zero slope (P>0.05). This relatively constant wave I trend suggests that the peripheral auditory system (PAS) including ME conduction, cochlear transduction and basic synaptic delay does not vary significantly at this age. There is general agreement for this behaviour in the literature on peripheral auditory maturation. It is likely, however, that there is some interaction between ME function and the days after birth of the testing. As Chuang *et al.*[12] noted, ME properties at this stage would be unlikely to have age-dependant maturation that could be observed within this time frame. Transient ME conditions are likely to mask any subtle changes due to maturational behaviour. Changes in cochlea performance have been reported[12, 13] during the preterm period. However, Geal-Dor *et al.*[14] reported that the inner ear matures earlier than the ME structures. In addition, Pujol *et al.*[15] reported that structural development of the cochlea is mature by 30 weeks GA. The functioning of the cochlea has been linked to thyroid hormone levels[13]. It should be noted that thyroid hormone levels may only be a marker for other factors. Fisher and Klein[16] reported the surge in thyroid levels occurs at 21 weeks GA, reaching half maximum levels by 26 weeks GA. So it would be unlikely that there is a rapid maturation of cochlea function during the term period.

Waves III and V both show a reduction during the term period. The wave III L-A function gradient is ≈-0.05ms/week with wave V displaying ≈-0.11ms/week. With the data available, these maturational relationships do not reach the required statistical level of probability (P>0.05). However, the increasing gradient progressing through the auditory system shows the behaviour expected, a compound effect. The overall wave V L-A function displays a slightly higher gradient than Gorga *et al.*[17] who found a value of -0.064ms/week during the term period. These results indicate that any changes in latency of ABR parameters is likely to be observed in the central auditory system beyond the cochlea.

The IPL measurements can provide valuable information on different parts of the auditory system beyond the cochlea. As stated in Chapter One, the I-V IPL can be used to eliminate the PAS structures preceding the auditory nerve. The I-III IPL is indicative of characteristics in the auditory nerve and cochlea nucleus. The III-V IPL represents the

lower brainstem between the cochlea nucleus and the lateral lemniscus. The data collected at the base stimulus rate is being used to assess neural transmission and basic synaptic delay.

The absolute latency data suggests a constant value for wave I with waves III and V displaying higher gradients. This suggests a predominantly central role in the reduction of latency values during the term period. The L-A function for the I-V IPL has a gradient of ≈-0.12ms/week. These results would suggest an increase in central transmission properties with GA during the term period. However, the lack of significance for the trend mean that the gradient cannot be quoted with certainty. This result does, however, support the behaviour indicated by the absolute latency data. The I-III IPL has a trend of ≈-0.08ms/week compared to the ≈-0.06ms/week of the III-V IPL. Again, the data available does not allow for this result to be statistically supported ($P>0.05$). This result does, however, suggest that both the auditory nerve (I-III IPL) and the lower brainstem region (III-V IPL) show a continuing evolution with GA during the term period. The relationship of these IPLs to each other can be expressed in terms of the I-III/III-V ratio. The trend data previously described should show a reduction of this ratio with age. Plotting the ratio from the raw data confirms this behaviour with an L-A gradient of ≈-0.02ms/week (one outlier removed). No significant difference in maturational rates for the I-III and III-V IPLs was found when assessed by the unpaired t test ($P>0.05$).

The L-I function for the absolute latency of wave V was also examined to assess neural sensitivity. Results reported in previous studies[18, 19] tend to be variable and conflicting making them of little use. The L-I function assesses the effect to latency of intensity levels. This intensity interaction can be used as an indicator of neural sensitivity. It should be noted that threshold levels can affect function gradients. Higher thresholds (20 or 30dB nHL) may eliminate prolonged values found with low intensity levels (ie. 10dB nHL). This has the affect of lowering the function gradient in subjects with 20 or 30dB nHL thresholds. This increases the degree of variability. The mean L-I function gradient for this group of term infants was found to be 0.350ms/10dB nHL (n=26 S.D. 0.150). This is in agreement with Stockard *et al.*[20] who reported a L-I function gradient of 0.36ms/10dB nHL for term newborns. The trend for the L-I function during the term period was found to be ≈-0.002ms/10dB nHL per week which is slight and not

significant (P>0.05). This would indicate that there is not a dramatic change in neural sensitivity during the term period when measured using the L-I function.

7.3.2 Extra-uterine Maturation of the Preterm Infant

The scatter plots for the preterm group can be seen in Appendix D. The one-sample t test was implemented to assess maturational trends against a zero slope. Unlike the term data, statistical significance was achieved for maturational processes for the majority of ABR parameters. This is probably due to the greater number of data points and the greater range of PCA. Absolute latency data for waves I, III and V all show significant maturational relationships (P<0.001) of reducing L-A functions during the preterm period. The III-V and I-V IPLs also show significant maturational L-A functions with values of P<0.001 and P<0.005 respectively. No significant difference from a zero gradient was found for the I-III IPL (P>0.05).

Conductive maturation of the PAS (wave I) is governed by characteristics of ME function, cochlear transduction maturation and enhancements in basic synaptic delay[5]. The data from this current study indicates a maturation in wave I latency of ≈-0.09ms/week during the preterm period. A maturational process is supported with the one-sample t test (P<0.001). This is in agreement with Krumholz *et al.*[1] who also reported -0.09ms/week. Stockard *et al.*[20] reported an increased L-A function gradient of -0.45ms/week up to 32 weeks PCA with a gradient of -0.15ms/week from 32 weeks to term. These findings confirm a maturational process during the preterm period for the conductive properties of the PAS.

Maturation of the conductive properties of the ME system during the preterm period remains poorly defined in the literature. As mentioned, Chuang *et al.*[12] reported that transient ME conditions during the preterm period would mask any age-dependant maturational process. Studies on otoacoustic emissions[21, 22, 23] have shown a maturation of cochlea performance during the preterm period. It is likely that a combination of increased cochlear transduction and a decrease in the basic synaptic delay would create the reduction observed. No maturational process was noted in the tympanometric data acquired for the preterm infants on this study. This supports the suggestion of no

age-dependant maturational process for ME function. This aspect will be further discussed later in this chapter.

Krumholz *et al.*[1] reported maturation of -0.16 and -0.17ms/week for waves III and V. There is a general consensus of ≈-0.2ms/week for the clinically used wave V latency[24]. However, the results for this current study indicate maturation of ≈-0.09 ($P<0.001$) and ≈-0.16ms/week ($P<0.001$) for waves III and V respectively. Whilst the data for wave V is in agreement with the other studies, there is a discrepancy with Krumholz *et al.*[1] data for the wave III reduction in latency. The similarity of the maturation for waves I and III for the data from this current study suggests zero maturation for the I-III IPL. However, the Krumholz *et al.*[1] data would indicate approximately zero maturation for the III-V IPL, both waves III and V displaying similar maturation. However, studying their IPL data shows that this is not the case, this would question the validity of their wave III data.

Krumholz *et al.*[1] suggest maturation for I-III, III-V and I-V IPLs of -0.07, -0.06 and -0.09ms/week respectively. Logic would dictate that the summation of maturation for the individual parts of the brainstem should approximate the overall brainstem measure (I-V IPL). This behaviour was noted by Krumholz *et al.*[1]. The IPL data for this current study indicates maturation of +0.005 ($P>0.05$), ≈-0.06 ($P<0.001$) and ≈-0.07ms/week ($P<0.005$) for the I-III, III-V and I-V IPLs respectively. This data equates well and is statistically supported. These trends are also in agreement with the behaviour suggested by the absolute latency maturation (zero maturation for the I-III IPL).

The L-I function (wave V) for the preterm infants displays a L-A function of -0.0022ms/10dB nHL per week. This is slightly higher than the term variation with GA of -0.0015ms/10dB nHL per week. This slight increase over the term value is probably due to the increase variability before 34 weeks PCA. Although the relationship is not statistically supported, it is in agreement with Stockard *et al.*[20]. They reported that newborn L-I gradients are higher than those of adults, also that preterm infants have steeper gradients than term newborns. The trends for this study would support a reducing L-I gradient during the preterm period. In addition, that the maturation in neural sensitivity for the preterm infants is decreased at later PCAs. These results would suggest some increase in neural sensitivity during the preterm period. Whether preterm

L-I functions are comparable with term infants during the term period will be examined in Section 7.5 on the effects of the preterm birth.

The results from this current study indicate that the preterm PAS goes through a maturational process in it's conductive properties. This occurs at a rate of ≈-0.09ms/week. This behaviour is statistically supported ($P<0.001$). This would be chiefly due to changes in cochlear transduction and basic synaptic delay. Clear maturational characteristics in the ME during this period are not currently defined in the literature. This is supported by the maturational characteristics, or lack of, in the tympanometric data of Chapter Six. This will be discussed further in the Section 7.5 on the effects of the preterm birth. No consistent trend was found for the term group. This is to be expected with the increased scatter of the data due to the number of subjects and the errors involved in GA estimation.

The current study also indicates a maturational process of neural transmission during the preterm period in the central auditory pathways (I-V IPL). However, the contribution of the auditory nerve (I-III IPL) to this maturational behaviour is minimal. The difference in maturational characteristics between the I-III IPL (indicative of the auditory nerve and cochlea nucleus) and the III-V IPL (indicative of the lower brainstem region) is statistically supported by the unpaired t test for the two slopes ($P<0.02$). It is proposed that maturation of neural transmission is concentrated in the lower brainstem region, and occurs at a rate of ≈-0.06ms/week. Both this result and the indication of an overall central maturation are statistically supported ($P<0.001$ and $P<0.005$ respectively). This result could support the theory that myelination (the predominating factor in neural transmission) occurs in a centripetal direction. Comparison of the I-III IPL data with term data will indicate whether the auditory nerve is indeed already mature (to term newborn standards) or whether maturation has yet to occur.

The reductions observed with GA for the term infants suggest that both the auditory nerve and lower brainstem have increasing neural transmission. This is supported by the overall I-V IPL and the relative latency reduction of the absolute latency of wave V compared to wave I. The lack of significance for these trends means that these relationships cannot be quoted with certainty. Further testing beyond the term

period would be necessary to assess whether these characteristics show true maturational behaviour that will continue beyond the term period.

7.4 Rate Effect (37 and 61pps)

Readings taken at 13pps display transmission properties of the auditory system. Readings taken much above 20pps will be subject to the same time delays caused by transmission characteristics. In addition, it has been suggested that the nerve fibres have insufficient time to fully re-energise. This creates an additional delay which is related to the stimulus rate[25]. The latency prolongation with rate is more pronounced for less mature auditory systems. It can be used as an assessment tool for auditory adaptation. As previously mentioned, synaptic efficacy is being considered as the dominant factor affecting adaptation. The additional data was collected using stimulus rates of 37 and 61pps.

The prolongation of latency is not strictly linear when plotted against stimulus rate (in pps). This study has consistently found the rate effect between 37-13pps to be greater than between 61-37pps. Data was collected at three stimulus rates; 13, 37 and 61pps. These correspond to time intervals between stimuli of 77, 27 and 16ms respectively. It can be seen that, although the stimulus rates are proportional, the recovery time is much reduced above 13pps. This is why it has been reported[26] that the major alteration in latency occurs before 20 or 30pps. It is assumed that, if a neural generator requires less than 77ms to fully recover, then testing at 13pps will not induce a rate effect.

As previously mentioned, the rate effect is being used to assess synaptic efficacy. This assumption was discussed in Chapter One. It is possible that there is interaction between other mechanisms for the rate effect. It is assumed that synaptic efficacy is the predominant factor. When referring to maturational process in synaptic efficacy it is important to note that this could involve establishment of new synaptic connections as well as development of existing established connections.

Maturation of the rate effect has received relatively little previous attention compared with the standard recordings at low stimulus rates. Commonly, the only reference to rate effects is the L-R function for the absolute latency of wave V. Whilst

this parameter behaves in a similar manner to other rate parameters, it is less reliable due to the linear regression construction using only three data points. The use of just three data points with this method is not ideal. There is also disagreement on the relationship between stimulus rate and the subsequent latency shift. Jiang *et al.*[27] reported this relationship to be linear, Lina-Granade *et al.*[28] and the results of this current study would suggest a non-linear relationship. Considering that synaptic recovery is dependant on the time interval between successive stimuli (1/pps), there is no reason why this relationship should be linear. The results from this current study indicate a greater effect between 13 and 37pps than between 37 and 61pps. For this study, the shift for the 61-13pps parameter was found to be the most robust measure. Subtraction of the low stimulus rate data with this parameter also removes the conductive component.

7.4.1 Intra-uterine Maturation of the Term Infant

The rate effect on wave I is essentially constant during the term period. This measure is an assessment of the synapse present in the hair cells of the cochlea. It has been reported[28] that there is no evidence of a maturational process for this synapse or that detection is not possible. Lina-Granade *et al.*[28] also reported a compound effect of rate attenuation progressing through the auditory system. This would support the lack of identification of a rate attenuation maturational process for wave I.

Maturational processes for waves III and V for rate attenuation are observed. These waves show a reduction with GA of -0.037 and -0.043ms/week for the 61-13pps shift for waves III and V respectively. These trends would indicate a greater maturation for the auditory nerve (I-III IPL) over the lower brainstem region (III-V IPL). IPL data supports the presence of a maturational process in the central system beyond the cochlea. The I-V IPL displays a maturation in rate attenuation of ≈-0.05ms/week for the 61-13pps shift. The absolute latency characteristics are further supported by the I-III and III-V IPL behaviour. Whilst the I-III IPL displays a maturational trend (≈-0.06ms/week), the III-V IPL shows a relatively constant trend (≈-0.01ms/week) for the 61-13pps shift.

The lack of maturation in 61-3pps shift for wave I and the III-V IPL is in agreement with the lack of significance from a zero slope ($P>0.05$). However, the I-III IPL shows a good level of significance ($P<0.005$) of a maturational trend. This supports

the suggestion that change in rate attenuation occurs in the auditory nerve and not in the lower brainstem region. This indicates that the term infant has a continuing maturation of the synaptic index assessed with the rate effect in the auditory nerve.

7.4.2 Extra-uterine Maturation of the Preterm Infant

There is agreement in the literature that a reduction of the rate effect exist during the preterm period[29, 30]. Data tends to concentrate on the L-R function of the absolute latency of wave V. Studies of the rate effect on IPL parameters are more limited. Lina-Granade *et al.*[28] studied the rate effect on various parameters in the preterm period. However, a discussion of maturation was not presented. They presented data in four time categories over the preterm period. Data for waves I and III, and the I-III and I-V IPLs display inconsistent maturational characteristics. Only the III-V IPL and the absolute latency of wave V show consistent reductions in rate attenuation. Only the wave V reduction was found to be statistically significant. This data will be discussed further in the following section on mean rate effect data.

The 61-13pps parameter will be discussed here due to its robust nature. The L-A functions (61-13pps) for this current study support the findings of Durieux-Smith *et al.*[4] who reported no age interaction for wave I, but considerable interaction for waves III and V. The L-A functions for this current study are +0.005, ≈-0.05 and ≈-0.07ms/week for waves I, III and V respectively. Ken-Dror *et al.*[31] indicated wave III, as well as wave I, to be independent of age. This would suggest an absence of synaptic development in the I-III IPL region.

As with the term results, the preterm trend for the rate attenuation (61-13pps) of wave I is constant. This would suggest either that the maturation of the synaptic properties under rapid stimulation has already taken place or that it is yet to occur. This matter will be addressed in Section 7.5 on the effects of the preterm birth. The relative rate shifts of both term and preterm infants will be compared.

Waves III and V display maturational trends of -0.050 ($P<0.005$) and -0.065ms/week ($P<0.005$) respectively for the 61-13pps shift. As with the term data, the greater reduction is indicated between waves I and III. The central nature of maturation of the rate effect is confirmed by the I-V IPL with a rate of ≈-0.05ms/week ($P<0.05$) for

the 61-13pps shift. This value is the same as that observed for the term infants during the term period. This could suggest that central maturation of the synaptic rate index in the preterm infant occurs at the same rate as that observed with GA in the term infant. The actual values of the rate effect will be discussed in Section 7.5.

Observing the I-III and III-V IPLs, again the central maturation is concentrated in the auditory nerve (I-III IPL). Trends for the 61-13pps shift are ≈-0.06 ($P<0.005$) and ≈-0.01ms/week ($P<0.05$) for I-III and III-V IPLs respectively. This confirms the relationship indicated by absolute latency parameters. These results again support the suggestion that the maturational process concerned with the synaptic index measured by rate attenuation is concentrated in the auditory nerve region. The lower brainstem region displays an evolution at a rate approximately four times less than that for the auditory nerve. Both PAS and lower brainstem regions show relatively constant data.

Both term and preterm infants display no significant maturation of rate attenuation characteristics for the PAS. Both groups suggest a central maturation from the absolute latency data. This is supported in both cases by the I-V IPL. The preterm I-V IPL 61-13pps shift trend is statistically significant ($P<0.05$). It is also noted that for both term and preterm infants the great majority of reduction in the 61-13pps shift values is located in the I-III IPL auditory nerve region. Both groups display ≈-0.06ms/week for the I-III IPL compared to the ≈-0.01ms/week for the III-V IPL.

The similarity of maturational behaviour between the term and preterm infants could lead to the suggestion that the synaptic index measured by rate attenuation is independent of term or preterm birth. However, it is necessary to study the actual values of the 61-13pps latency shift to be able to conclude the actual effects of the preterm birth. This will be discussed in the next section.

7.5 Effects of the Preterm Birth

To assess the effects of the preterm birth, data collected from preterm infants during the term period will be compared with the normative term infant data. For the purpose of this comparison, data from infant LOB (on the preterm study) will be excluded, this is due to this infant being born small-for-dates and not prematurely. The test PCA distributions were examined to ensure that any effects identified are not due to

different distributions. This is especially important due to the extended PCA range of the term group, these infants being tested up until 42 weeks PCA. Mean test PCAs for the term and preterm groups are 39^{+2} (S.D. 1^{+3}) and 38^{+3} weeks (S.D. 1^{+1}) PCA respectively. The unpaired Wilcoxon test showed no significant ($P>0.05$) difference between term and preterm test PCAs.

7.5.1 Previous Studies

It is of interest to establish the effect of the preterm birth on auditory characteristics by the term period. There are three main areas of difference for infants between term intra-uterine and preterm extra-uterine environments. These are nutrition, environmental acoustic stimuli and physical dimensions. Considering first the nutritional aspects of the preterm birth. The importance of appropriate nutritional supplies was highlighted in Section 1.5.3 with further details of the dietary aspects of this thesis being discussed in Chapter Four. Poor nutritional content during the rapid development of the preterm period can affect the myelination process and the structure of cells vital to neurological function and integrity. It has been reported[32] that nutrition plays an essential role in dietary lipids which are intimately connected with development of myelin. Myelin incorporates EFAs which cannot be synthesized within the body. EFAs must be obtained by external nutrition. The supply comes from the mother for the baby in utero and then from breastmilk after birth. The requirement for EFAs is high soon after birth. The nutritional involvement in synaptic efficacy has not been reported in detail. Obviously, an appropriate nutritional supply is essential for good formation of synaptic connections and generation of chemical transmitter substances (Section 1.5.3). The nutritional differences between intra- and extra-uterine environments during the preterm period is clear. The intra-uterine nutritional supply is provided directly from the mother's blood through the placenta. The extra-uterine diet is from breast or formula milk. The feeding regimes do not replicate the nutritional composition of the mother's blood. The natural nutrition provided by the mother's blood is essential for intra-uterine maturation. A nutritional supply is dependant on the transfer of nutrients across the placental barrier. The extra-uterine environment, whatever feed is used, will not presently replicate the nutrition provided by the mother's blood supply.

The other factor to consider is the interaction of acoustic stimuli present in the extra-uterine environment. It is possible that a certain amount of early acoustic stimulation from the normal environment is beneficial to the auditory system. Pasman *et al.*[33] presented hypotheses for the differences observed between term and preterm infant ABR characteristics. They presented the hypothesis that the extra-uterine preterm exposure may lead to enhanced auditory performance, this being reflected in shorter latencies and IPLs. However, the level of noise (60-80dB nHL) produced by an intensive care incubator over a long period of time is unlikely to be beneficial to the immature auditory system of the preterm infant. Pasman *et al.*[33] also presented the alternative hypothesis that extra-uterine preterm exposure leads to delayed development of the auditory system, this being reflected in prolonged latencies and IPLs of the ABR.

The results of studies investigating intra- and extra-uterine maturation tend to give variable and conflicting conclusions. Some studies[34, 35] find no difference in ABR latencies and IPLs for term and preterm infants tested at the same PCA. Delorme *et al.*[36] and Collett *et al.*[37], however, reported shorter latencies for waves III and V, and lesser IPLs (I-III and I-V) for preterm infants with an extra-uterine life >2 weeks, compared to infants with <2 weeks extra-uterine life at the same PCA. This would indicate a beneficial effect with the extra-uterine environment leading to enhanced auditory performance. Eggermont and Salamy[5] reported prolongation of waves I, III and V latencies in preterm infants compared to term infants at the same PCA. They reported no difference in all IPLs. This would indicate that prematurity, and the following period in the extra-uterine environment, would result in poorer PAS performance when tested during the term period. Their IPL characteristics would suggest that there is no difference in central auditory properties.

Pasman *et al.*[33] studied preterm and term ABRs at low stimulus rate (11pps) during the term period. They concluded that there was no difference in ABR morphology between the two groups. They suggested a tendency for prolonged latencies for all ABR components in the preterm group. However, these results were not statistically significant.

Whilst myelination increases the conductile neural properties, there are other possible factors affecting ABR latencies with increasing age. Obviously, as an infant grows so head size will increase resulting in an increase in the length of the auditory

pathways. In addition, it is also recognized that axonal diameter also alters with growth. Lengthening of the auditory pathways does not affect the number of nodes present along an axon. With the process of saltatory conduction the variation in internodal distance would not affect transmission properties to a significant extent. It would, thus, be expected that this characteristic would not be a significant component when observing ABR data.

Moore *et al.*[38] studied ABR conduction times and correlated them with a reconstruction of the auditory pathways from post-mortem foetal data. They studied infants ranging from the preterm period up to 1 year of age. For this current research, changes from the early preterm period through to term are of interest. Moore *et al.*[38] suggested that more than one aspect of the myelination process is responsible for the apparent increase in conduction time in the auditory pathways. The fact that reductions in conduction time are observed during the preterm period suggests that any increase in path length is more than balanced by changes in axonal structure or process characteristics. Moore *et al.*[38] commented on the previous research into increasing peripheral nerve length and its affect on conduction velocity. They reported the behaviour of axonal lengthening where internodal distances increase with the number of nodes on the axon remaining the same. They concluded that the saltatory conduction behaviour of the axon would make the conduction velocity independent of internodal length. It would, therefore, be possible to conclude that increasing length of auditory pathways would not affect ABR latencies.

It has already been stated that generally reductions in ABR latencies at low stimulus rate are predominantly due to increased thickness of myelin around the axon. An increase in axonal diameter due to the development of the infant is also indicated. Colello[39] reported that these two processes are connected with myelin forming oligodendrocytes displaying control over increases in axonal diameter. It is, therefore, possible that increasing axonal diameter could be an additional minor component of the overall decrease in ABR latencies that are linked to the myelination process and the basic synaptic delay.

Ponton *et al.*[40] investigated the considerable variability of central IPL parameters with infants. This tends to be greater for the preterm infant. Ponton *et al.*[40] examined the influence of head size on the variability observed with IPLs. They normalized IPL data

with head circumference. Normalization was found not to reduce IPL variability. They found that there was a negative correlation between IPLs and head circumference that varied with PCA. Before 42 weeks PCA, this behaviour was observed for the III-V and I-V IPLs, and not for the I-III IPL. They concluded that increasing neural transmission in the auditory nerve and lower brainstem compensate (I-III IPL), or overcompensated (III-V and I-V IPLs), for lengthening of the pathways and increases in head circumference. It is thus concluded that head size, with the association of path length, does not present a major component affecting neural transmission when compared with the combination of the myelination process and the considerable basic synaptic delay component.

7.5.2 Current Study Results

7.5.2.1 Base Stimulus Rate (13pps)

Considering the absolute latency values, all waves show prolonged means during the term period for the preterm over the term group. Wave I has a latency mean of 2.42ms (n=17 S.D. 0.374) for preterm compared with 2.17ms (n=28 S.D. 0.309) for the term group (0.25ms difference). Wave III means are 5.23 (n=17 S.D. 0.384) and 5.06ms (n=28 S.D. 0.419) for preterm and term groups respectively (0.17ms difference). Wave V displays means of 7.94 (n=17 S.D. 0.458) and 7.48ms (n=28 S.D.0.374) respectively (0.46ms difference).

The discrepancy observed with wave I would suggest a difference in the conductive properties of the PAS in favour of the term infant. This discrepancy was tested with the unpaired Wilcoxon test and was found to be statistically significant ($P<0.05$). The increased discrepancy for wave V would indicate a possible difference in the central system. This discrepancy was also found to be significant ($P<0.05$). These results agree with the data of Pasman *et al.*[33] and Krumholz *et al.*[1]. Both tested using low stimulus rates with preterm and term infants. The GA criteria for Pasman *et al.*[33] was <34 weeks GA (tested at 70dB nHL) with Krumholz *et al.*[1] using a criteria of the first test being prior to 35 weeks PCA (tested at 65dB nHL). It should be noted that Krumholz *et al.*[1] preterm data is for 37-38 weeks PCA. Absolute latency values are indicative of the auditory system up to the particular wave. The differences observed in

waves III and V will thus include the apparent difference observed for wave I. Wave I is a measure of the PAS conduction and the basic function synaptic delay from the synapse near the OHCs.

Eggermont and Salamy[5] attributed this behaviour of absolute latencies to be predominantly due to the high incidence of ME effusion in the preterm infant. This produces mild conductive problems in the ME. The differing status of the preterm ME throughout the preterm period (including term period data) is supported by the tympanometric data collected for this current study. Tympanometric data did not show a maturation of the more complex tympanometric data during the preterm period. Preterm data collected during the term period was still found to be more complex. The preterm ME status was found to have a greater degree of mass control with poorer conductive properties than the term data.

The discrepancies identified by Pasman *et al.*[33] and Krumholz *et al.*[1] for wave I are 0.19 and 0.33ms respectively (both in favour of term infants). The PAS discrepancy for this current study is 0.25ms. The reduced conductile properties of the ME in the first few days after birth has been widely reported[41, 42, 43]. However, the ME of the preterm infant has had a significant period of time in the extra-uterine environment for normalization after birth. This result would appear to be caused by the high rates of transient ME conditions observed in the preterm population. It has been reported[41, 42] that ME effusion and other ME conditions are highly dependant on incubation and the inactivity of the preterm infant. It is unlikely that there is any significant affect from cochlear transduction. Fisher and Klein[16] reported the surge in thyroid hormone levels occurs at 21 weeks GA, reaching half maximum levels by 26 weeks GA. Thyroid hormone levels have been strongly linked with cochlea function[13], this could suggest an early functional maturation in the cochlea. The difference is probably due to the ME function and possibly basic synaptic delay.

The discrepancies for waves III and V are reported as being similar in value. Pasman *et al.*[33] suggested 0.12ms and Krumholz *et al.*[1] reported ≈0.45ms for both waves III and V. The wave V discrepancy (0.45ms ($P<0.05$)) for this current study is in agreement with Krumholz *et al.*[1]. Pasman *et al.*[33] concluded that there was a tendency for increased absolute latencies for the preterm group. However, they did not find these differences to be significant. Krumholz *et al.*[1] did not test for significance between term

and preterm data. The data for this current study shows significance for the discrepancy in absolute latency for wave V ($P<0.05$). In order to eliminate the apparent difference in PAS function between term and preterm infants the IPL parameters need to be examined.

The I-III IPL means show no difference between term and preterm infants. There was no statistical evidence of a difference ($P>0.05$). Means for term and preterm infants are 2.80 (n=17 S.D. 0.486) and 2.85ms (n=28 S.D. 0.361) respectively. This was suggested by the absolute latency data with the wave III difference being less than that observed for wave I. This result indicates that the transmission properties of the auditory nerve are similar for both term and preterm infants (preterm only 0.05ms less). This similarity is confirmed by data from Krumholz *et al.*[1] who reported a 0.01ms difference between term and preterm infants. Pasman *et al.*[33] reported a 0.07ms discrepancy. It has previously been stated (Section 7.3.2) that the preterm infants display a zero gradient for the I-III IPL L-A function. However, it was not clear whether the stationary maturational state of the preterm data showed a lack of maturation which would result in deficient performance in this region or whether maturation had already taken place and was already performing to term standards. The lack of difference in I-III IPLs for term and preterm infants during the term period indicates that the transmission properties of the auditory nerve are mature at an early stage in the preterm period.

Ponton *et al.*[40] investigated the I-III IPL by studying the I-II IPL as a measure of myelination status and the II-III IPL as a synaptic measure. The assumptions for their model were discussed in detail in Section 1.6. Their data shows some reduction in latency for the I-III IPL. However, their measure of axonal transmission (I-II IPL) shows no significant maturation. They conclude that changes in the I-III IPL are due to the II-III IPL component reducing during the preterm period. They suggest that this IPL is indicative of synaptic function in the I-III IPL region. Whilst the maturation of the I-III IPL for this current study shows no maturational process at the base stimulus rate, the Ponton *et al.*[40] data may indicate that a synaptic maturation will be observed in the rate effect data for this current study. This will be discussed later. As previously stated, myelination occurs in a centripetal direction[15], delayed myelination will, thus, affect later ABR components to a greater extent.

The discrepancy in means for the III-V IPL (0.25ms) for this current study would suggest a tendency for prolonged values in the preterm infant. However, the data

available did not reach the required statistical level of probability (P>0.05). Some MRI studies[44, 45] have found no discernible difference in myelin status between preterm and term infants during the term period. Huppi *et al.*[46] reported that the increase in myelination of preterm infants replicates the intra-uterine myelination process as studied from autopsy studies. Ponton *et al.*[40] studied the III-IV IPL to assess transmission properties of the lower brainstem and the IV-V IPL to examine synaptic delay. Their data show a maturational pattern for the III-IV IPL with a lesser variation observed for the IV-V IPL. This would indicate that the majority of reduction observed in the III-V IPL is due to axonal transmission properties rather than improvements in basic synaptic function delay. Krumholz *et al.*[1] reported a discrepancy of only 0.10ms with Pasman *et al.*[33] reporting the same means for both term and preterm infants. The results of this current study suggest that the lower brainstem region would be more susceptible to delayed myelination in the preterm infant than the auditory nerve.

The I-V IPL displays the combination of the discrepancies observed for the I-III and III-V IPLs. The term mean is 0.18ms lower than that of the preterm group. This value is supported by Krumholz *et al.*[1] who also reported a 0.18ms difference in favour of term infants. Pasman *et al.*[33] reported just a 0.07ms difference. The result from this current study supports the tendency observed for the lower brainstem region. However, this discrepancy cannot be statistically supported with the data available (P>0.05).

The mean L-I function (13pps), reported as corresponding with neural sensitivity, shows a higher mean for the preterm group. Means are 0.464ms/10dB nHL (n=17 S.D. 0.096) for preterm compared with 0.350ms/10dB nHL (n=28 S.D. 0.150) for the term group. This difference does not reach the required statistical level of probability (P>0.05). Threshold levels are the same for term and preterm infants during the term period. There is, therefore, no interaction of threshold levels which can alter L-I function data[c]. This shows a tendency for greater neural sensitivity (when assessed with the L-I function) for the term infant before the wave V generator. This would suggest that the extra-uterine environment of the preterm infant has not been beneficial to the development of neural sensitivity. These results support the concept that the L-I function

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[c] It has previously been noted that differences in threshold level can modify L-I function gradients. This is not a problem for the term period data being analyzed.

displays higher values for preterm infants during the preterm period, and that a maturational process is present.

In summary, the three important measurements are the absolute latency of wave I, and the I-III and III-V IPLs. These results indicate a prolonged PAS response in the preterm infant, even when compared to term infants tested in the first few days after birth. This is probably due to the characteristics of the ME in the preterm infant. The relatively long period of preterm life before the term period testing does not eradicate the more complex ME characteristics in the preterm population. This is confirmed by tympanometric data for the preterm infants during the term period where more complex patterns are still observed. They show a greater mass dominance with lesser conductive properties.

The auditory nerve, which shows no maturation in the transmission properties during the preterm period, has the same characteristics for both preterm and term infants. This would indicate that the transmission properties of the auditory nerve are not unduly affected by the extra-uterine environment during the preterm period. There is a tendency for prolonged latencies in the lower brainstem region in the preterm infants. This could indicate that the extra-uterine environment has led to delayed development in this region. It does suggest that the lower brainstem region would be more susceptible to delayed development than the auditory nerve. It cannot be stipulated as to whether this is due to myelination characteristics or basic synaptic delay.

7.5.2.2 Synaptic Efficacy

The rate induced shift in means between 13 and 61pps data will be examined with the mean values for the 61-13pps shift. The 61pps data was found to be more reliable and less variable than the 37pps data. A full list of mean data at all three stimulus rates and the 61-13pps shift can be seen in Table 7.1. Statistical significance for differences between term and preterm data were not found for parameters at 61pps ($P>0.05$). Greater numbers would be required in order to demonstrate the small differences involved with the rate effect to be significant. A difference for the 61-13pps parameter was found to be statistically significant for wave III ($P<0.05$).

The maturation of the rate effect has been given relatively little previous attention compared to the low stimulus rate testing commonly used for myelination assessment. Lina-Granade *et al.*[28] tested preterm infants between 32 and 39 weeks PCA. The tests were performed once only between 2 days and 11 weeks after birth. Stimulus rates of 20, 41.3 and 61.3pps were implemented. The authors used the assumption that subtraction of low rate data from higher rate data would eliminate the neural transmission component. However, the authors do not justify the use of a 20pps stimulus rate as their low rate baseline[d]. It has been reported[47], and this current study would agree, that the greatest rate effect is observed between 10 and 30pps. It is, therefore, possible that 20pps data will be affected to some extent by rate. Lina-Granade *et al.*[28] arranged their data in four age groups to 39 weeks PCA (preterm infants). However, their comparison is with adult data and not a term sample. Their data is, thus, only useful for analysis of the relationship between the various parameters[e].

Lina-Granade *et al.*[28], however, reported inconsistent parameter relationships between age categories. At 36-37 weeks PCA, their 61.3-20pps shifts are similar for waves I and III, with the wave V shift being considerably increased. At 38-39 weeks PCA, they show a more equal spacing for the shifts for waves I, III and V. Their IPL data for 36-37 weeks PCA shows the 61.3-20pps shift to be the same for the I-III and III-V IPLs, with the I-V IPL shift being greater. At 38-39 weeks PCA, the I-III IPL shift is greater than the III-V shift. The preterm data for this current study (36-40 weeks PCA) for the 61-13pps shift display a slight increase from wave I to wave III. The wave V shift is much increased. This behaviour is replicated in the term data for this study.

Considering the rate induced shift on wave I means, it can be seen that there is a greater effect for the term infants. The shift between 13 and 61pps means is 0.41ms (19%) for the term infants compared to a preterm shift of 0.32ms (13%). The rate effect measured by the 61-13pps shift shows a similar relationship. Means for this parameter are 0.414ms (n=28 S.D. 0.376) for term infants with preterms displaying 0.349ms (n=17 S.D. 0.286). Whilst these discrepancies are not significant ($P>0.05$), they do indicate that the synapse located near the OHCs of the preterm infant does not have impaired performance when compared to term data.

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[d] There is general agreement in the literature to use 10-13pps.

[e] Values are lower than those for this current study due to the higher baseline stimulus rate of 20pps. It is assumed that this rate was used to reduce testing time.

Wave III displays a similar pattern, with term infants displaying a mean shift (61pps) of 0.58ms (11%) compared with 0.39ms (8%) for preterms. This is reflected in the 61-13pps means which are 0.576 (n=28 S.D. 0.346) and 0.374ms (n=17 S.D. 0.206) for term and preterm infants respectively. The data for the 61-13pps shift shows significance for this difference ($P<0.05$). This could indicate a greater synaptic efficacy for the preterm infants in the auditory nerve region.

The clinically used wave V shows a slightly larger rate induced mean shift for the term group. The shift is 0.84ms (11%) for the term group with the preterm group displaying a 0.74ms (9%) shift. The 61-13pps means are 0.846 (n=28 S.D. 0.307) and 0.780ms (n=17 S.D. 0.410) for term and preterm respectively. This result is confirmed by the mean L-R function for wave V at 60dB nHL. The preterm group have a slightly lower mean L-R gradient (155μs/decade, n=17 S.D. 81) compared with the term value (176μs/decade, n=28 S.D. 64). This difference is not statistically significant ($P>0.05$) and might suggest a lessening in the discrepancy from wave III. This could indicate a greater difference in the I-III IPL.

Eliminating the PAS (wave I), the I-III IPL shows a rate induced mean shift of 0.20ms (7%) for terms with preterms being affected by only 0.07ms (3%). Again, this is confirmed by the 61-13pps means which are 0.176 (n=28 S.D. 0.369) and 0.011ms (n=17 S.D. 0.338) for term and preterm infants respectively. This result displays a very low rate effect for the preterm group. This, again, indicates that the preterm auditory nerve region synaptic efficacy is not impaired by the extra-uterine environment. This result shows that the concentration of synaptic index maturation (Section 7.4.2) in the auditory nerve region leads to appreciably better synaptic index values for the preterm infants during the term period.

The rate induced mean shift on the lower brainstem region (III-V IPL) is actually greater for the preterm infants at 0.36ms (13.3%). The term group show a 0.23ms (9%) shift. This was suggested by the absolute latency data. The greater rate effect for the preterm group is supported by the 61-13pps means, these being 0.406 (n=17 S.D. 0.400) and 0.262ms (n=28 S.D. 0.314) for preterm and term respectively. The lack of a maturational process for the III-V IPL mentioned in Section 7.4.2 would appear to result in less mature synaptic index values by the term period. This result indicates that the

lower brainstem may be more susceptible to any synaptic deficiency resulting from the preterm birth.

The measure of the complete central system (I-V IPL) displays similar rate induced mean shifts for both term and preterm data. Both show mean shifts of 0.46ms, this being a 9% increase for the term group, and 8% for the preterm group. This is supported with 61-13pps mean values of 0.456 (n=28 S.D. 0.413) and 0.428ms (n=17 S.D. 0.524) for term and preterm respectively. These results would suggest that the synaptic efficacy of the overall central system (measured by the I-V IPL) is the same for both term and preterm infants during the term period.

The use of rate attenuation for production of a synaptic index measure has been utilized here to identify the effects of the extra-uterine maturation process on the synaptic efficacy of the preterm infant. The characteristics of the results from this current study indicate that the synaptic efficacy is not unduly affected by maturation in the extra-uterine environment. The rate attenuation data suggests lower values for the 61-13pps shift for the preterm group for all parameters except the III-V IPL lower brainstem region.

It is unlikely that any physical dimensional differences would affect the synaptic index measured by rate attenuation. Nutritional involvement may contribute to the poorer synaptic efficacy of the lower brainstem region. The nutritional supply to the preterm infants (as a group) would not be optimal compared to the intra-uterine supply of the term group. The greater synaptic efficacy observed in the auditory system preceding the brainstem might indicate the involvement of acoustic stimulation from the extra-uterine environment.

It is concluded that the lower brainstem region would be most susceptible to any possible synaptic deficiency. In addition, that the synaptic efficacy of the system preceding the brainstem is not unduly affected by the extra-uterine environment. A greater sample size is required to enable a more conclusive result for the lower brainstem region.

7.6 Gender Effects

7.6.1 Base Stimulus Rate (13pps)

Minor gender effects have been found in adults, females displaying shorter IPLs with wave V latency being approximately 0.2ms shorter than males[48]. However, some studies[49, 50] have reported no gender differences for normal infants and preadolescent children. Results regarding the preterm infant are also variable. Cox *et al.*[51] reported gender differences in infants during the 33 to 39 weeks PCA period. They found these differences to be transient, testing of the same subjects at 4 months PCA produced no gender difference. These differences have been suggested as being sequalae of the higher incidence of neurological and risk factor interaction seen with the preterm male[52].

It has previously been reported by Murray[53] that the central auditory system (I-V IPL) shows a significant gender difference in the preterm infant. Murray[53] reported that the female I-V IPL mean is 0.25ms less than the male. Work by Beiser *et al.*[54] suggested that the gender differences found with the central system are not due to physical dimensional differences. They found no difference in skull diameter. Murray[53] suggested that the gender differences are not due to risk factors, female infants on that study having significantly higher Obstetric Complication Scale optimal scores. An alternative hypothesis is that, if female newborns are physically more mature than males by 4-6 weeks at birth[55], then they could also be more neurologically mature.

Eldridge and Salamy[56] also reported evidence of gender effects for preterm infants on their study. They observed shorter latencies for waves III and V for female infants. They suggested that reported[57, 58, 59] reasons for lesser latency values in females, such as head size and differences in anatomical distances between major auditory structures cannot explain all the gender effects observed. They also reported no birthweight, GA or assisted ventilation correlations. Previous research suggests that there might be a gender effect in the preterm infant. This effect being predominantly on a central level. The IPL measures are, thus, of particular interest. The transmission and synaptic properties of absolute latency data will be discussed briefly.

No gender effects were found for the term infants on this current study for all absolute latencies and IPLs at the base stimulus rate. The unpaired Wilcoxon test shows no cases where differences exceed the required significance level ($P<0.05$). This suggests

that the intra-uterine maturation is gender independent at low stimulus rates. This supports previous work by Jacobson *et al.*[60] who suggested the absence of gender effects for term infants.

Wave I L-A functions for the preterm group display similar gradient and value for both genders with confidence bands overlapping. However, data during the term period shows significantly ($P<0.05$) lower values for the female infants. This suggests poorer performance for the PAS of male preterm infants. This measure represents the conductive properties of the ME and transduction through the cochlea. Preterm wave V data displays a tendency for prolonged means for male infants from 28 to 40 weeks PCA. There is evidence of this in the L-A function behaviour. However, both confidence bands again overlap and is thus not significant. Obviously, this absolute wave V latency will include some delay from the difference observed for wave I.

The I-V IPL shows limited conclusive evidence of a central gender effect. Females have lower means before 38 weeks PCA, and L-A functions show males with higher values. However, these trends were not found to be significantly different when tested with the unpaired t test ($P>0.05$). There is also no significant difference for gender during the term period when tested with the unpaired Wilcoxon test ($P>0.05$). There is no gender difference for the auditory nerve (I-III IPL) and only a slight difference for the lower brainstem region (III-V IPL).

In conclusion, these results confirm the lack of gender interaction on the transmission properties in the term infant. There is a possible gender difference for the PAS of the preterm infant during the term period. It is possible that this result is due to the greater risk factors observed with the male preterm infants[61]. This could result in a greater time spent in the NICU and in incubation leading to a higher incidence of transient ME conditions. There is no evidence of a gender difference for the transmission properties of the auditory nerve, and only a slight difference observed for the lower brainstem region. This might indicate that any possible difference would be located in the lower brainstem region. A greater number of subjects would be required in order to demonstrate the small differences to be significant. There is no detectable gender interaction on neural sensitivity, when measured by the L-I function, for both term and preterm infants.

7.6.2 Rate Effect (61pps)

Data at 61pps and the 61-13pps shift were found to the more robust parameters when investigating rate attenuation. The wave I data for the term group show a lesser mean for female infants for the 61-13pps shift (≈0.18ms difference). This difference was not found to be significant (P>0.05). There was no suggestion of a difference for wave I in the preterm infants during the preterm period or by the term period. The term infants wave V 61-13pps shift shows similar behaviour to that of wave I with the female mean being ≈0.27ms lower than the male. This behaviour is repeated for the preterm data with confidence bands being separate. There is statistical support for a difference by the term period (P<0.05).

The I-V IPL 61-13pps shift for the preterm infants is in agreement with the suggestion of a central gender effect with consistently lower means for the female group. However, L-A function data is inconclusive. The term period I-V IPL shift does not support the absolute latency data showing no significant difference (P>0.05). Both mean data and L-A functions shows no gender effect for the synaptic index of the auditory nerve (I-III IPL) for both term and preterm infants. The lower brainstem measure (III-V IPL) shows a lesser mean for the female term infants (≈0.17ms) for the 61-13pps shift. This is supported by a significant gender difference for the 61pps measure (P<0.05). As with the term data, the 61pps measure shows a gender effect for the lower brainstem region (III-V IPL). However, the variability observed with the 61-13pps measure does not allow for any gender effect to be identified for the lower brainstem region. These results could, however, indicate that any possible central gender difference is likely to be located in the lower brainstem region.

7.7 Dietary Analysis

Some difficulties were encountered with placement of subjects into the dietary groupings. The criteria for the dietary groupings was >75% volume of any particular feeding regime[f]. A number of infants did not have >75% of any one particular feed. The

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[f] As previously stated, dietary intake is being measured by volume as precise energy intake calculations are not possible for EBM. The target level of 75% was stipulated by the sponsors.

combination of EBM and formula milk was found with most infants. In total, nineteen infants met the dietary grouping criteria. The control formula group (fed formula not enriched with long chain polyunsaturated fatty acids (LCPUFA)) was found to have a non-uniform test PCA distribution. This, in combination with low sample size (n=5), created unreliable L-A functions. The other dietary groups; Prematil formula infants (fed formula enriched with LCPUFAs) and breastfed (EBM) infants had equal sample sizes (n=7). They displayed more consistent maturational information. The analysis is, therefore, concerned with the Prematil and EBM groups. It is of interest to assess the relative auditory characteristics between Prematil formula fed (enriched with LCPUFAs) and EBM infants.

It has previously been reported[62, 63] that infants fed formula milks containing 'significant' levels of LCPUFAs will have similar visual maturation characteristics as infants fed breastmilk. It is, therefore, of interest to compare the relative maturation between Prematil and EBM fed infants. Makrides *et al.*[62] also reported that infants fed formula devoid of LCPUFA enrichment would display poorer or more variable visual maturation characteristics. It is suggested that diet can also influence the maturational characteristics of the auditory system.

Further information on the subjects tested for diet can be seen in Section 5.7. Regression coefficients of L-A functions were compared using the unpaired t test. The unpaired Wilcoxon test was implemented on the term period data to assess the effects of nutrition during the preterm period on auditory functional status by the term period. Blood analysis was performed in conjunction with the ABR assessment. Samples were collected for each test session. These were then analyzed for LCPUFA content. The levels of enrichment in the Prematil formula was relatively low. The blood analysis did not show a significant difference in LCPUFA levels between the Prematil formula and EBM fed groups.

The ABR assessment is concerned with the interaction of dietary regimes on the auditory system. Since nutrition will affect the auditory system on a neurological level, the IPL measures are of particular interest.

7.7.1 Base Stimulus Rate (13pps)

Wave I category means show greater values for the Prematil than for EBM infants. This is seen from 28 to 40 weeks PCA. The difference is ≈0.20ms from 32 weeks PCA onwards. L-A functions for wave I are in agreement with the mean data and have confidence bands not overlapping. The EBM mean during the term period is lower than that of the Prematil group, but this apparent difference is not significant ($P>0.05$). Wave III shows similar means for EBM and Prematil infants from 32 weeks PCA onwards. L-A functions are similar with overlapping confidence bands.

Wave V displays greater mean values for the Prematil group from 28 weeks PCA onwards. The difference is ≈0.32ms for the 36-40 PCA period. Again, this difference does not achieve the required statistical level of probability ($P>0.05$). The difference in mean values between wave I to wave V (from the absolute latency data) would suggest a discrepancy of ≈0.10ms in favour of the EBM infants. By similar reasoning, the Prematil group should display lesser means for the I-III IPL. The III-V IPL should display lesser means for the EBM group.

The central system (I-V IPL) displays L-A functions that are similar in gradient and value for all dietary groups. The EBM mean is ≈0.10ms less than that for Prematil by the term period. This difference is observed in the category mean data from 32 weeks PCA. This is in agreement with the absolute latency data. This difference is very slight and is not significant ($P>0.05$). This indicates that, for the central auditory system, both Prematil and EBM have similar neural transmission characteristics. However, the auditory nerve and lower brainstem regions (I-III and III-V IPLs) show larger differences between Prematil and EBM infants. Both EBM and Prematil L-A functions do not display significant maturational trends ($P>0.05$). The Prematil term period mean is ≈0.21ms lower than the EBM infants for the I-III IPL auditory nerve measure. Category mean data for the III-V IPL shows lesser values for the EBM infants throughout the preterm period. This is also the case during the term period where the EBM mean is lower by ≈0.31ms. This confirms the behaviour suggested by the absolute latency data. The data available does not reach the required level of probability ($P>0.05$) for any conclusions to be drawn.

The inconsistent differences observed with the I-III and III-V IPLs with PCA

indicate that no consistent dietary interaction can be identified in the maturational time course between the Prematil and EBM infants. In order for any of the minor discrepancies to be supported a greater number of subjects would be required to achieve significance. It has to be concluded that the central auditory system neural transmission maturation is the same for both dietary groups. This is displayed with the I-V IPL central measure.

7.7.2 Rate Effect (61-13pps)

The 61-13pps shift mean values (waves I, III and V) during the 36 to 40 week PCA period show greater values for the EBM group than the Prematil infants. The differences are ≈0.08, ≈0.01 and ≈0.16ms for waves I, III and V respectively. The L-A functions do not indicate significantly different gradients or values. Whilst the discrepancies for waves I and III during the term period are low, the wave V result suggests that the region beyond wave III is of particular interest (ie. III-V IPL).

The 61-13pps means for the I-V IPL for Prematil and EBM infants show similar values (difference of ≈0.07ms). This would suggest that the central auditory system overall synaptic efficacy is the same for both diets. The auditory nerve (I-III IPL) also shows similar means for Prematil and EBM infants in the term period (difference of ≈0.07ms). Data for the lower brainstem region (III-V IPL) displays a lower 61-13pps mean for the Prematil infants during the 36-40PCA period. This difference is ≈0.15ms. This result accords with the difference observed with the absolute latency of wave V during this period. However, this apparent difference is not statistically significant ($P>0.05$).

The 61-13pps mean data for all parameters is not consistent throughout the preterm period. The 61-13pps shows considerable variation during the preterm period. To be able to support the small discrepancies observed a larger number of subjects would be required. This would aid demonstration of significance for small discrepancies and would minimize type I and II errors. These results would suggest that the Prematil formula (enriched with LCPUFAs) is comparable with the EBM group for the synaptic efficacy of the central auditory system (I-V IPL). The larger discrepancy found with the lower brainstem measure might suggest that this region is of particular interest for future

research.

7.8 Discussion of Tympanometry Results

A full set (226-2000Hz) of susceptance and conductance tympanograms were recorded from all 28 term infants and 8 of the infants on the preterm study. Testing was implemented after commencement of the preterm study. Testing was found to be well tolerated by both term and preterm infants. Obtaining a hermetic seal was found to be more difficult with incubated infants, due to the confines of the enclosed style incubators. However, the preterm infants were less restless during testing. The earliest test PCA was 29^{+5} weeks with infants being approximately 10 days old. A full set of recordings were achieved from the early PCA infants.

7.8.1 Effect of Successive Runs (Term Data)

The admittance (Y) tympanograms recorded initially, during, and at the end of the testing, enabled assessment of the effects of successive runs on the tympanometric data. The initial test (Y_1) shows that the term group are equally divided between the 1Y and 3Y patterns. By the eighth run (Y_8), 68% of these infants display the 3Y pattern. This result is repeated at the end of the test session (Y_{28}).

The pressure peak location for these admittance tympanograms was also assessed for successive runs. The mean shift between the first (Y_1) and the eighth (Y_8) run was -7daPa. The mean pressure peak location is seen to move to a slightly more negative value. Considering the fluctuating nature of this measure, this shift is minor. The static peak immittance values of these peaks increase between the first (Y_1) and eighth (Y_8) run in 61% of term infants. The remainder display a decrease. The shift in means between Y_1 and Y_8 is $\approx$0.06mmho.

These results indicate that some infants are affected by successive runs. The effect occurs predominantly within the first eight runs. Wilson *et al.*[64] reported the greatest effect to be between the 3rd and the 5th runs. Eighteen percent of infants who show a single peak admittance tympanogram on initial test will be modified to a multi-peaked pattern by the eighth run. This is probably due to the behaviour of both

susceptance and conductance data[64]. The change in static peak immittance value between these two runs is relatively minor when compared to the magnitude of the overall admittance data. These results, therefore, show that the effect of successive runs is not a problem in multiple tympanometric testing. Infants on this study were subjected to partial and full pressurizations before testing was commenced. These were performed for the validation of the hermetic seal, instrument calibration checking, and with restless infants. Vanpeperstraete *et al.*[65] found this procedure of performing 'dummy' runs to be advantageous in reducing the effects of successive runs.

7.8.2 Tympanogram Morphology

Tympanogram morphology at 226Hz is standard[g] in nature for term infants. Both susceptance and conductance patterns generally have clear peaks or troughs for static peak marking. At higher frequencies (678 and 1000Hz), notches become broader and less well defined. Both term and preterm data readily display asymmetric notching of the susceptance patterns at these higher frequencies. The conductance patterns remain more regular, but have asymmetric tail (extreme pressure) characteristics. Whilst the identification of pressure peak location for conductance data is relatively easy at the higher frequencies, the susceptance tympanogram requires more attention. The positive pressure notch shoulder for susceptance tends to be well defined. However, the negative pressure notch shoulder is often diminished or absent. These characteristics have been reported for the presence of cerumen on the ear canal wall[66] or the condition of external otitis media (EOM)[67]. However, the widespread presence of cerumen or EOM is not evident. This behaviour is further investigated in the discussion of the infant external auditory meatus (EAM). The patterns found with these infants are unusual, but repetitive characteristics are observed.

The preterm data also shows standard patterns at 226Hz, but these tend to be more complex in nature (more complex Vanhuyse classifications). At higher frequencies, the patterns become increasingly more complex than for their term counterparts. The poorer morphology of the preterm data at higher frequencies does not eliminate static

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[g] The standard tympanograms at low frequency are single peaked bell shapes, or with single sharp notching.

peak identification. However, appreciation of the distorted patterns is necessary. It would appear that the poor, or more complex nature of the preterm tympanogram is not age dependant throughout the preterm period. Holte *et al.*[68] suggested maturation of tympanogram morphology over the first month of life in the term infant. This is not the case for the preterm infant.

7.8.3 Vanhuyse Classification System

The Vanhuyse system of tympanogram classification was implemented to assess tympanogram complexity. This system was also used to investigate the difference between term and preterm data, and to observe maturational trends in the preterm infant. The Vanhuyse system classifies susceptance and conductance tympanograms by the number of extrema, various combinations are observed. A strict criteria was enforced for these classifications, with only clear sharp notching being accepted. The previously mentioned broader patterns, occurring at the higher frequencies, were not classified. It is necessary to distinguish between normal sharp notching and broader, more undulating patterns.

The majority of term infants (86%) show a 3B pattern at 226Hz. These infants are equally divided for the 1G and 3G patterns. There is a low incidence of 1B1G, 3B3G and 0B1G patterns, with all the term infants being classified (not as 'Other'). The 1B1G pattern is eliminated with the preterm infants at this frequency. The 5B pattern is more dominant than the simpler 3B pattern and the prevalence for 3G over 1G is increased. This indicates that the preterm 226Hz data will display more complex susceptance and conductance tympanograms than term infants. There would appear to be some evidence of a maturational trend from 5B to 3B patterns. However, the 5B pattern does occur at the later PCAs.

The more complex patterns of the preterm group (even at low frequency) indicate that, not only do all the preterm infants have resonant frequencies <226Hz, but that their resonant frequency is even lower than those term infants displaying resonance <226Hz. Patterns tend to be more complex above the resonant frequency. The more complex patterns found with the preterm group suggest that these infants are greater distanced from their resonant frequency than their term counterparts. This leads to the

proposal that the mass contribution is greater in the preterm infants. In addition, that this behaviour is found throughout the preterm period. The probable cause is ME effusion. This behaviour will be discussed further in the susceptance characteristics.

The 3B pattern is still dominant in the term infant at 300Hz with 82% displaying this pattern. However, at this frequency the 3G pattern is more prevalent (79% of term infants). Whilst there is a low incidence of the 1G pattern with the term infants, the 1G pattern is eliminated from the preterm data. There are no 1B1G patterns for either group. The difference between the term and preterm infants is predominantly with the susceptance pattern. The preterm group show a bias towards the 5B rather than 3B pattern. Again, this confirms that the 300Hz frequency is greater removed from the resonant frequency of the preterm infants than that of the term infants.

The most noticeable difference between groups occurs at 400Hz. The term group show 68% of infants being classified[h]. For preterm infants <32 weeks PCA, all patterns are classified as 'Other'. After 32 weeks PCA, 40% or more patterns are classified as 'Other'. Of those classified, 94% of term and all of the preterm infants display a 3G pattern. Again, the susceptance data shows more complex patterns for preterm infants. The 5B pattern is more prevalent than the 3B in the preterm infants from 34 weeks PCA onwards. The term group shows 58% (of those classified) with 3B and 42% with 5B.

The limit of usefulness would appear to be 400Hz. Beyond 400Hz (up to 678Hz), less than 20% of term patterns can be classified[i] (68% being classified at 400Hz). No preterm patterns can be classified (100% 'Other') beyond 400Hz. For clinical applications the limit would be 300Hz where ≈90% of infants can be classified.

7.8.4 External Auditory Meatus (EAM) Effects

The EAM characteristics in the adult are of little consequence. The ear canal acts as a hard-walled rigid cylinder providing minimal acoustic and mechanical interaction. This sub-system, characterized only by the volume of air within the ear canal, can be eliminated from data leaving only the ME system. Testing at extreme pressure, with the TM artificially stiffened, produces a susceptance value (stiffness of air volume) with zero

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[h] 32% being classified as 'Other'.
[i] Not classified as 'Other'.

conductance. This behaviour indicates that the ear canal element is cyclically storing and releasing energy without dissipation[69]. This characteristic produces a high admittance phase angle, confirming the basic assumptions underlying the tympanometric procedures being used.

The infant EAM, however, has been reported as suffering from distention under pressure application[68, 70]. This is due to the immature structures and tissues of the EAM. The tympanometric characteristic of this behaviour is a monotonic rise in susceptance with increasing pressure. This 0B1G pattern is not prevalent in this data. It is, therefore, concluded that ear canal wall distention with these infants is relatively minor (some interaction could be possible with particular subjects). Keefe and Levi[71] suggested that power-based rather than pressure-based responses could be implemented to assess the relative accuracy of compensated tympanometric measurements. They indicated that pressurization of the ear canal in young infants may lead to changes in canal volume.

The predominant characteristics found with this study are the asymmetry of the susceptance notch shoulders combined with asymmetric negative and positive tail values. These characteristics can be observed with the presence of additional matter in the ear canal (eg. cerumen)[66]. The presence of cerumen was not widespread in the infants tested during this study. The ear canal was observed for presence of cerumen and the probe tip observed after testing. It is proposed that the EAM characteristics of the neonate are responsible for the patterns observed. Whilst the diameter change with pressure application has previously been considered, the actual motion and acoustic properties of the EAM tissues have not been examined in detail in previous research. The flexibility and oedematous nature of the EAM tissues require them to be considered as active mechanical structures. The vibratory motion of these tissues will give rise to both stiffness and mass elements, as well as an acoustic absorption component (conductance). In order to examine the effects of the EAM, data at extreme pressure must be observed. With extreme pressure the TM is artificially stiffened, thus 'detaching' the ME system. Tail values are regularly used for compensation of data as previously mentioned. However, the actual behaviour of these tail values has received little previous attention. Sprague *et al.*[70] reported on the difficulty of distinguishing between characteristics of the ear canal and those of the actual ME system. In order to use tympanometry to assess ME function, it is essential to understand all the mechanical systems involved in producing

the tympanometric data. This thesis has advanced the discussion of the ME as a mechanical system in relation to tympanometric recording.

Both term and preterm infants display the negative tail phase angles characteristics which would be indicative of EAM interaction[j]. As previously mentioned, the vibratory motion of ear canal tissues can alter the stiffness and mass components of the tympanometric data. The addition of a conductance component from the ear canal is indicative of acoustic energy absorption. The motion and acoustical properties of the EAM would allow for these characteristics.

7.9 Static Peak Immittance

It was necessary to use peak locations at low frequency for identification of peaks at higher frequencies. Often the peak or trough location for the static measurements at the higher frequencies was actually a slight inflexion. With an appreciation of the characteristics of neonate high frequency data, static peak location can be successful. The pressure peak locations for all admittance components was generally at negative pressure. The incidence of extreme pressure location was very low. The majority of data shows values between -10 and -20daPa. A negative bias with infants is considered abnormal, but not pathological[72]. This indicates that the preterm birth, and the subsequent incubation, does not affect the ME pressure status in these infants.

7.9.1 Term Susceptance

The resonant characteristics, as indicated by the susceptance data, for the term infants suggest that 50% have resonance below 226Hz. This implies the interaction of a lower stiffness or greater mass contribution, or a combination of both. The results from this current study suggest that 93% of term infants will display resonance characteristics below 400Hz. Meyer *et al.*[73] studied a single term infant and found a resonant frequency between 300 and 400Hz for the first 60 days after birth. At higher frequencies there is a definite prevalence of stiffness control. At 678Hz, 97% display positive susceptance

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[j] With only susceptance (and near zero conductance) the admittance phase angle will be >80°. This would be expected with adult data.

(stiffness). By 1000Hz, 20% of term infants again show negative susceptance (mass) data. Static peak values at these higher frequencies should be observed with care, the patterns tend to be more complex and erroneous data more common.

These results suggest that a 678Hz probe tone (with the majority of term infants showing a stiffness dominated system) would be more sensitive to tympanometric assessment than the standard 226Hz. However, as previously mentioned, the tympanogram patterns found at 678Hz are less standard in nature than those encountered at lower frequencies. The 678Hz probe tone would be recommended for tympanometric assessment of the term infant.

7.9.2 Preterm Susceptance

The preterm susceptance data at 226Hz displays negative static peak values for all subjects before 33 weeks PCA. Beyond 33 weeks the majority of data is still negative. In addition, all subjects initially displaying positive values become negative with age. This shows a definite bias towards mass controlled systems in the preterm infant at 226Hz. A maturational trend towards a greater stiffness contribution is not observed. This would be expected, as 50% of term infants are stiffness dominated. The greater mass dominance of the preterm infant would indicate a lesser stiffness or greater mass contribution. This stiffness behaviour is possible with the more immature structures and tissues of the preterm ME system. The increase in mass is probably related to the presence of ME fluid. However, the lack of a maturational trend, to term-like characteristics, over the considerable time period involved is interesting. This indicates a continuing effect of the preterm birth on ME status throughout the preterm period.

At 678Hz, there is a greater prevalence of positive stiffness data. A number of infants display very low values that fluctuate between positive and negative. At 1000Hz, only three infants on initial test, display mass controlled susceptance. All become stiffness dominated by the next test session. There are no clear maturational trends at these higher frequencies. Variations with PCA are observed, with some subjects increasing and some decreasing in positive susceptance values.

These results suggest that static peak susceptance in the preterm infant does not display consistent maturational trends. Intersubject variability is high, this would suggest

that the factors involved are highly influenced by the individual transient conditions and not by the maturation of ME structures.

7.9.3 Conductance Characteristics

Static peak conductance means are lower for preterm than term infants for frequencies below 1750Hz. The discrepancy increases slightly between 226 and 678Hz. The difference is far greater at 1000Hz. This suggests that there is less energy flow through the preterm ME conductive elements. The EAM properties are eliminated by the compensation procedure. It is ,therefore, suggested that these differences are due to TM or ME properties. It is possible that there is some interaction of ME dimensional characteristics. This supports the previous suggestion of superior ME function (with a lesser mass contribution) in the term infant.

7.10 Term Period Testing of Preterm Infants

Of the seven preterm infants tested during the term period (36-40 weeks PCA), five display mass control at 226Hz, with two showing approximately zero susceptance (suggestive of resonant frequency). This indicates that for tympanometric sensitivity, 226Hz is not appropriate for testing of preterm infants during the term period. Whilst the majority of term infants are stiffness controlled at 678Hz, the preterm data still shows incidence of mass control. At 1000Hz, 100% have stiffness controlled systems. This suggests that the higher frequency is beneficial in achieving stiffness dominance in the preterm infant during the term period. However, the morphology of both susceptance and conductance tympanograms at 1000Hz is of concern. There are high levels of asymmetry and the possibility of obtaining erroneous data is increased. It would be suggested that both 226 and 678Hz probe tones be implemented for assessment of the preterm infant during the term period. The observation of more complex, and more variable data would be expected.

7.11 Impedance Data

The impedance components are useful in assessing notching in susceptance tympanograms. Notching occurs when the resistance component is greater than the negative reactance. Term infants show mean resistance being much greater than negative reactance for frequencies below 500Hz. Means between 500 and 1000Hz show similar values. From 1000Hz the resistance mean is again considerably greater. This behaviour at high frequency supports the distorted notching seen with the susceptance data.

At low frequency (226Hz), the resistance-reactance relationship should invert at approximately 1000 hours (≈42 days) after birth in the term infant[74]. The preterm data was investigated for this maturational characteristic. The majority of subjects were tested beyond 42 days after birth, but no inversion of the relationship was observed. This implies that the maturational processes of the preterm ME status are slower than those seen with the term infant. This supports the earlier findings that there is not a maturational trend to term-like characteristics over the period of this study.

7.12 Tympanometry and the ABR

Tympanometric data recorded from both term and preterm infants was presented in Chapter Six. This data has been further examined in this chapter. It was noted that whilst some studies[75, 76] have reported on the possible correlations between tympanometry and OAEs, the technical links between tympanometry and the ABR during the preterm period has received limited attention. This current study has investigated complex multi-frequency admittance tympanometry in the term and preterm populations. The preterm infants were tested in conjunction with the ABR recording to assess maturational characterises during the preterm period. Eight preterm infants were tested with full ABR and tympanometry test protocols throughout the preterm period.

The ABR parameter of interest is the PAS wave I parameter at the base stimulus rate. This is used as an assessment of the conductive properties of the PAS. Factors involved in the system include ME function, cochlear transduction and basic synaptic delay. A maturation of this measure during the preterm period was noted in the results of this current study. A reduction in latency of ≈-0.09ms/week was statistically

confirmed ($P<0.001$). This indicates an increase in the overall conductive properties of the PAS. As previously discussed with the ABR results, this reduction in latency is most probably due to the cochlear transduction and basic synaptic delay components. Chuang *et al.*[12] reported that changes observed in ME function due to transient conditions would mask any age dependant maturational characteristics. Studies[12, 13] have reported maturation of cochlea performance through OAE testing during the preterm period.

Geal-Dor et al.[14], however, reported that the cochlea's structural development is mature by 30 weeks GA. Fisher and Klein[16] also reported that the function of the cochlea is connected to surges in thyroid hormone levels. They suggest that the major surge occurs at 21 weeks GA with half maximum levels being reached by 26 weeks GA. It is not possible to distinguish the contributions of basic synaptic delay on the reduction observed in wave I. No maturational trend was observed for rate attenuation of wave I during the preterm period. This suggests no change in the effects of rapid stimulation on the synapse in the PAS. This does not necessarily indicate stationary behaviour for the basic synaptic delay. It is thus obvious that the exact maturation of cochlea function, including synaptic delay, is not presently defined.

In order to study the affect of ME maturation on the ABR wave I parameter the tympanometric data collected during the preterm period is of interest. Whilst it is not possible to directly link numerical data from the ABR and tympanometric data, it is of interest to examine maturational trends in the tympanometric data. It should be noted that the most reliable tympanometric data collected from the neonate population is at low frequency (ie. 226Hz). This is obviously a different stimulus from the click stimulus presented for ABR collection (ie. broadband noise 500-2000Hz).

The characteristics of tympanometric data in individual preterm infants was discussed in Sections 6.12 and 6.13. It was reported that the preterm infants had a greater degree of mass control in the ME system when compared with the normative term data. The maturational characteristics revealed some infants having increasing mass contribution with others displaying the opposite behaviour. The data showed a lack of a definite maturational process. This indicates changing ME status with transient conditions. The suggestion of masking from TM characteristics was also presented in Sections 7.3.1 and 7.3.2.

The interesting finding of the preterm tympanometric characteristics is the

continuing complexity of data into the term period. Comparison of preterm and term infant data during the term period still indicated poorer conductive performance in the preterm group. This lack of a maturational process in tympanometric data during the preterm period suggests that the maturation of the ABR wave I parameter would not be due to ME properties. Cochlear transduction and basic synaptic delay would be the major components responsible for the changes observed.

The poorer conductive properties of preterm infants, as shown by the tympanometric data, supports the increased wave I latency mean of the preterm infants during the term period compared to the normative term data. The preterm wave I latency mean during the term period was found to be 0.25ms greater than that of the term group. This difference was found to be statistically significant ($P<0.05$) when tested by the unpaired Wilcoxon test. The tympanometric data indicates that the ME would contribute to the discrepancy observed in the ABR data.

The difference between preterm and term tympanometric data is unlikely to be due to TM or ear canal effects. With the considerable period of time in the extra-uterine environment of the preterm infants the properties of the TM and ear canal would have normalized by the term period. It is proposed that ME function is responsible for the differences observed. This would indicate that ME status of the preterm infant has an affect on ABR wave I latency during the term period.

Table 7.1 Comparison of Term and Preterm means (S.D.) n

Parameter	TERM				
	13pps	37pps	61pps	Mean 61 - mean13 (%)***	61-13pps
I	2.17 (0.309)28	2.43 (0.550)28	2.58 (0.521)28	0.41 (18.9)	0.414 (0.376)28
III	5.06 (0.419)28	5.45 (0.437)28	5.64 (0.441)28	0.58 (11.5)	0.576 (0.346)28
V	7.49 (0.374)28	7.99 (0.492)28	8.33 (0.503)28	0.84 (11.2)	0.846 (0.307)28
I-III	2.85 (0.361)27	3.02 (0.385)28	3.05 (0.431)28	0.20 (7.0)	0.176 (0.369)27
III-V	2.46 (0.270)27	2.54 (0.315)28	2.69 (0.327)28	0.23 (9.3)	0.262 (0.314)27
I-V	5.31 (0.477)28	5.55 (0.439)28	5.77 (0.469)28	0.46 (8.7)	0.456 (0.413)28
L-I*	0.350 (0.150)26	-	-	-	-
L-R**	176 (64)28	-	-	-	-

Parameter	PRETERM				
	13pps	37pps	61pps	Mean 61 - mean13 (%)***	61-13pps
I	2.42 (0.374)25	2.60 (0.391)26	2.74 (0.437)26	0.32 (13.2)	0.349 (0.286)25
III	5.23 (0.384)26	5.46 (0.350)26	5.62 (0.360)25	0.39 (7.5)	0.374 (0.206)25
V	7.94 (0.458)26	8.34 (0.831)26	8.68 (0.474)25	0.74 (9.3)	0.780 (0.410)25
I-III	2.80 (0.486)25	2.86 (0.410)26	2.87 (0.352)24	0.07 (2.5)	0.011 (0.338)24
III-V	2.71 (0.366)26	2.88 (0.270)26	3.07 (0.323)25	0.36 (13.3)	0.406 (0.400)25
I-V	5.49 (0.519)25	5.74 (0.424)26	5.95 (0.473)24	0.46 (8.4)	0.428 (0.524)24
L-I*	0.464 (0.096)26	-	-	-	-
L-R**	155 (81)24	-	-	-	-

*measured in ms/10dB nHL

**measured in μs/decade

***refers to the mean shift (and percentage increase) between 61 and 13pps data

37-13 and 61-13pps refer to rate differences in raw data

CHAPTER EIGHT

CONCLUSION

8.1 The Auditory Brainstem Response (ABR)

ABR data was successfully collected from 22 infants born <32 weeks GA (or 1500g). Initial testing was performed in the enclosed style incubators, in some cases within the first week after birth. The earliest test was during the 29th week PCA, with the subject being 10 days old. The majority of preterm ABRs had well defined morphology with clear peaks for marking. Waves I, III and V were found to be present at these early PCAs. Twenty-eight term infants were also tested using the same ABR protocol.

Preterm ABR data was found to be more variable than that seen with the term infants. This led to relatively large standard deviations for all data. This, combined with the low number of infants recruited, made it difficult to reach the required statistical level of probability for significance. This is a problem commonly encountered when testing the preterm population. The low number of subjects recruited onto the preterm study is due in part to the strict birth criteria (<32 weeks GA or <1500g) required by the sponsors.

Blood chemistry analysis did not show significant blood composition differences for the dietary regimes. This is probably due to the relatively low quantity of long chain polyunsaturated fatty acids (LCPUFA) added to the test formula. The quantity was restricted for safety reasons.

The data was analyzed to provide normative maturational data for the preterm population. The effect of gender on both term and preterm infants was investigated. The preterm data collected during the term period was compared to the normative term data. This provided an assessment of the difference between intra- and extra-uterine maturational processes.

Maturational characteristics were examined using linear L-A functions for grouped data. These were implemented for all ABR parameters (waves I, III and V, and associated IPLs) at the various stimulus rates (13, 37 and 61pps) and for rate shift data (eg. 61-13pps). Trends displaying maturational behaviour were identified with the

one-sample t test. This process was implemented with the preterm group to assess the maturational characteristics of the different parts of the auditory system.

Base stimulus rate (13pps) data was used to assess the conductive properties of the PAS and the neural transmission properties of the central auditory system. Neural transmission was assumed to be predominantly governed by myelination status and basic synaptic delay. The rate effect was used as a measure of synaptic efficacy, rate attenuation being increased for more immature synaptic function. The L-I function, the affect of intensity on latency, was used as a measure of neural sensitivity.

The PAS shows an improvement in its conductive properties during the preterm period. It is proposed that the major components of this improvement are cochlear transduction and basic synaptic delay. The tympanometric data from this study indicated a lack of maturation for the conductive characteristics of the middle ear (ME). There also exists a maturational process for neural transmission in the central auditory pathways as a whole. However, the contribution of the auditory nerve to this observed maturational behaviour is minimal. The difference in maturational time course between the auditory nerve and the lower brainstem region is statistically significant ($P<0.05$). It is thus concluded that the reduction observed in the clinically used absolute latency of the wave V parameter is due to maturational processes in the PAS and lower brainstem region. There is no significant maturational process for neural transmission in the auditory nerve during the preterm period.

The most robust rate attenuation parameter was found to be the 61-13pps shift. This parameter eliminates the measure at the base stimulus rate. There was found not to be a significant reduction in the rate effect for the PAS during the preterm period. However, there was indication of improvements in the rate effect for the central pathways. The greatest improvement was observed in the auditory nerve. Only a slight improvement was noted for the lower brainstem region. This indicates a greater developmental process for synaptic efficacy, as measured by rapid stimulation, in the auditory nerve.

The actual effects of the preterm birth on auditory function was investigated by comparing preterm infant data collected during the term period with the normative term infant data. This analysis shows the resulting characteristics of auditory maturation in the extra-uterine environment during a period of intense development. The greatest

difference between this environment and the intra-uterine environment afforded to the term infant is nutrition and the physical properties of the extra-uterine environment.

The preterm infant presents poorer conductive properties of the PAS when compared with newborn term infants. Tympanometric data from this study indicated poorer ME function for preterm infants throughout the preterm period. This behaviour persists into the term period where the preterm infants were still found to have poorer ME conductive properties than term infants tested just a few days after birth. The auditory nerve, which displays no maturation in its neural transmission characteristics during the preterm period, displayed the same properties for both term and preterm infants. This indicates that the lack of maturation in neural transmission properties of this region is replicating the stationary characteristics of the term infant developing in the intra-uterine environment. The preterm infants, however, display evidence of poorer neural transmission in the lower brainstem region during the term period. This could indicate that the maturation observed during the preterm period is not as great as that of the term infant in the intra-uterine environment. It cannot be stipulated as to whether this poorer neural transmission in the lower brainstem region is due to myelination status or basic synaptic function delay.

Synaptic efficacy was also assessed for differing function between the preterm and term infants. Rate attenuation was actually found to be less for the preterm infants tested during the term period than that observed for the term infants for the PAS and auditory nerve. Slightly higher levels of rate attenuation were observed in the preterm population than term for the lower brainstem region. This indicates that the synaptic efficacy (as measured by the rate effect) of the system preceding the brainstem is not unduly affected by the extra-uterine environment. This could be associated with the acoustic stimulation encountered during the preterm period in the extra-uterine environment. A greater sample size is required to enable a more conclusive result for the lower brainstem region. However, it is proposed that the lower brainstem region would be the area most susceptible to any possible synaptic deficiency. This could be caused by nutritional aspects of the preterm birth.

The conductive properties of the PAS show no gender difference for the term infants. However, a significant difference is observed for the preterm infants during the

term period. The female subjects display superior characteristics. This suggests poorer PAS performance for male preterm infants. This could be linked to the greater risk factors observed with male preterm infants which could result in greater time in the NICU and in incubation. There is no evidence of a gender difference for the auditory nerve transmission properties for the preterm infant. A slight difference was noted for the lower brainstem region. However, a greater number of subject would be required to demonstrate this small difference to be significant ($P>0.05$). No central neural transmission gender effect was found for the term group. There was also no detectable gender interaction for neural sensitivity, as measured by the L-I function, for both term and preterm infants.

There was no significant evidence for a gender effect for rate attenuation for the PAS for both term and preterm infants. This was also found to be the case for the overall measure of the central system. This lack of a central gender difference is confirmed for the auditory nerve. However, both term and preterm groups show evidence of a difference for the lower brainstem region. A greater number of subject would be required in order to show that differences observed with the 61-13pps shift are significant. These results indicate that any possible gender interaction with the rate effect would occur with the lower brainstem measure. In addition, that this could occur in term as well as the preterm population.

The dietary analysis was hampered by the low number of subjects in each group. The criteria for dietary groupings was >75% volume of any one particular feed. Mothers of preterm infants were encouraged to breast feed their infants. This often resulted in the infant receiving a combination of preterm formula and breast milk. Of those infants classified by diet, the control formula group (not enriched with LCPUFAs) was found to be unrepresentative. Analysis was thus concentrated on the comparison between breastfed and test formula (enriched with LCPUFAs) fed infants.

Maturational trends and category mean data for the PAS show EBM infants to have lower values than Prematil infants throughout the preterm period. This is also the case during the term period, but the difference does not reach the required statistical level of probability ($P>0.05$). Maturational trends for the central auditory system display similar behaviour for all dietary groups. This could indicate EBM and Prematil fed

infants to have similar central neural transmission properties.

Data for the auditory nerve and lower brainstem region show superior properties for the Prematil and EBM groups for these regions respectively. This shows inconsistent behaviour and does not reach the required level of probability ($P>0.05$). Greater numbers of subjects would be required for these results to be quoted with certainty.

There was not found to be any significant difference between dietary groups for rate attenuation of the PAS. Both Prematil and EBM groups display similar rate effect characteristics for the overall measure of the central auditory system. This is also the case for the auditory nerve measure. The Prematil group displays a lower mean that the EBM group for the lower brainstem region. However, this apparent difference is not statistically significant ($P>0.05$). The value of this difference, though not statistically significant, might suggest that the lower brainstem region would be of particular interest for future research into dietary interaction on the rate effect.

It would thus be proposed that infants fed EBM or formula containing LCPUFAs (with this particular composition) will have similar auditory function by the term period. The levels of LCPUFAs added to the formula milk for this study were conservative and this might indicate that higher levels are required in order to identify a measurable, significant benefit. It is concluded that breastmilk would be most beneficial to the preterm infant, than this particular formula feed, due to the similar auditory function and the addition medical advantages of breastmilk for the infants health.

The results discussed in this thesis add to the knowledge in relation to the use of the ABR with the term and preterm neonate populations. The dietary analysis has provided a platform for further research into the effects of the preterm diet on the maturation of the auditory system. The study has identified a number of additional areas where further research would be valuable. A summary of the principal ideas for further development can be seen in Chapter Nine.

Further research into the complex physiology of hearing is required to fully apply these results to the auditory system. These results are interpreted on particular physiological assumptions, and relies on the use of regions within the auditory system. The identification of the neural generators of the ABR would allow particular structures of the auditory system to be linked to wave morphology. This would enable a more

detailed application of these results.

8.2 Tympanometry

Tympanometry was successfully implemented with term and preterm infants. Obtaining an hermetic seal was more difficult with preterm infants due to the confines of the enclosed style incubators and the nature of the external auditory meatus (EAM). The preterm ear tends to be smaller in diameter. However, the relatively large number of tympanometric tests were well tolerated. Complete data sets were recorded from all infants tested. Successive run effects were minor, this was probably aided by the pressurizations performed prior to testing.

Notched susceptance tympanograms are present in both term and preterm infants at low frequency (226Hz). Term infants display 50% with mass dominance at this frequency, with 100% of preterm infants being mass dominated. Where mass dominance exists, infants will show poor sensitivity to tympanometry. This frequency (226Hz) is commonly used with infants due to its popularity in adult testing.

At 678Hz, 97% of term infants and the majority of preterm infants display stiffness dominance. This would increase the sensitivity to tympanometric testing. However, the patterns found at this frequency are more complex. At this frequency, an appreciation of neonate tympanogram characteristics is necessary for interpretation. Even though complexity is increased there are normative patterns. Utilization of a 1000Hz probe tone would not be advisable in the neonate population due to the interaction of the ear canal. This also affects the simpler, more standard patterns of the conductance tympanogram.

The Vanhuyse system of tympanogram classification is only useful for low frequency measurements. It is possible that a greater clinical application could be developed with this system for the neonate population. The modifications to Vanhuyse classifications for various pathologies needs to be investigated. This system was used to examine the more complex nature of the preterm tympanometric data, this occurred even at low frequency. The more complex data of the preterm infant does not show a maturational process; this complexity continues throughout the preterm period. This behaviour is confirmed by testing of the preterm infants during the term period. A greater

mass influence is still observed. This indicates that the maturation of tympanometric data observed in previous studies for the term infant is not present in the preterm infant. It is important that this is remembered when assessing preterm infants during the term period.

The infant ear canal does not provide the baseline data produced by the adult ear. The ear canal suffers from distention under pressure application. However, the characteristics observed with data from this study are suggestive of more interaction than just ear canal diameter variation. It is proposed that the ear canal tissues behave as active acoustic and mechanical systems. These results suggest that the oedematous nature of the ear canal tissues absorb sound energy increasing the conductance at extreme pressure. In addition, it is suggested that the ear canal wall tissues will have some vibratory motion. This will also modify the stiffness and mass components of the susceptance measurement. This behaviour needs to be fully understood to enable further clinical application.

Considering the maturational differences between the term and preterm infants, the resistance-reactance relationship of the impedance data does not show the maturational characteristics suggested by other studies for the term infant. These characteristics have not previously been investigated in the preterm population. This result further indicates that the maturational time course of tympanometric characteristics in the preterm infant are different from their term counterparts.

This research has investigated the mechanical aspects of the ME system in the term and preterm neonate populations. Normative data has been established for tympanogram shape and various numerical parameters. This thesis has examined how the different parts of the auditory system (including the EAM) influence the tympanometric response. The EAM has been established as a source of variability. The understanding, and appreciation, of various interactions is required to advance the usefulness of tympanometry in the neonate population.

Diagnostic capabilities of complex admittance tympanometry is currently limited. It tends to be used with little regard to the differences which exist between adult and infant anatomy. This thesis has laid the foundations for further research and possible clinical application. Future work on pathological ears and the preterm population is required. A summary of areas for further research are presented in Appendix G.

8.3 Tympanometry and the Auditory Brainstem Response (ABR)

There was an overall increase in the conductive properties of the PAS measured by the ABR during the preterm period. There were no maturational characteristics observed with the preterm tympanometric data. This would suggest that the ME conductive properties are not age-dependant in the preterm infant and remain affected by transient conditions. This indicates that the major contribution to the improvement observed with the ABR are due to the cochlear transduction and basic synaptic delay components.

The effect of the preterm birth was assessed by comparison with normative term data. This displayed prolonged ABR PAS conductive properties for the preterm group. This is supported by the more complex mass dominated characteristics of the preterm tympanometric data continuing into the term period. This indicates that ME function contributes to the poor ABR PAS measures. It also indicates that the considerable amount of time in the extra-uterine environment for the preterm infants has not been beneficial in the resolution of ME conditions occurring from birth. The preterm infants display poorer function compared to term infants tested only a few days after birth.

CHAPTER NINE

FURTHER WORK

9.1 Collaboration of Current Work

This present study has been successful in investigating the maturational characteristics of the preterm ABR and the effects by the term period of the preterm birth. The numbers of subjects recruited was limited by a number of constraints. The most limiting factor was the birth criteria set by the sponsors (<32 weeks GA or < 1500g birthweight). There are approximately 30 babies meeting this criteria born each year at Hillingdon Hospital. In addition, this population has relatively high levels of medical complications which do not allow for recruitment or involve specialist treatment at alternative centres. These aspects further affect recruitment as parental consent is required. For the most beneficial data testing must start within a week or so after birth. This is obviously a difficult time for the parents of infants born in this situation. It is thus not possible to recruit the majority of infants with the appropriate birth criteria.

Greater numbers of subjects would aid analysis of the results. An increased sample size would allow greater statistical power where small differences were observed. This could lead to significance being achieved and would also minimize type I and II errors. It would be especially useful for results achieving statistical levels of probability between P=0.1 and P=0.05. A major multi-centre study would be necessary in order to achieve the required number of subjects in a reasonable amount of time. This has added complications for organization and standardization of testing. There would also be a greater financial burden. Alternatively, a retrospective Meta analysis could be implemented if the test protocol was aligned with previous studies. Obviously, this is not possible when investigating dietary intake.

This present study utilized a broad test protocol for both ABR and tympanometric testing. This was necessary in order to establish useful ABR parameters for a comprehensive investigation of the maturational characteristics. The tympanometric testing was broad due to the lack of previous research into multi-frequency

multi-component testing with the preterm population. It would be beneficial to reduce these test protocols using the results of this present study as a guide to the most informative and robust parameters. This would help reduce testing time and could possibly allow for weekly rather than fortnightly testing. This would be beneficial to further study of maturational characteristics.

Further research is required into the interaction between long-chain polyunsaturated fatty acids (LCPUFA) and hearing function in the preterm infant. The test formula milk utilized for this study was enriched with a relatively low level LCPUFA composition. The levels were set by the sponsors and were limited by commercial and safety aspects. Results from the blood analysis study showed no detectable difference between diets on LCPUFA composition in the blood. It is thought that much higher levels are required to alter blood composition. It is proposed that a blood chemistry study should be implemented, without auditory testing, in order to assess methods and levels of LCPUFA enrichment. Following a successful trial of this nature, a full ABR study could then be implemented. A reduced test protocol could be introduced to minimize test times. The results of this current study could be utilized to identify the most useful and robust parameters for use in subsequent testing with the preterm population. A reduced test time would be beneficial in examining a larger number of subjects. The feeding regime criteria (>75% volume of a particular feed) needs to be revised as the majority of mothers opted initially for breast feeding, this regime being supplemented with formula where necessary. Supplementation with formula was generally required. However, not all the infants achieved the required level of any one particular feed. This impacted on the numbers for the dietary groupings.

Nutritional intake was measured by volume for this study. It would be beneficial to calculate the energy intake for each feed and the amount of LCPUFA composition being received. This is possible with formula milks but is uncertain for EBM. LCPUFA composition would have to be assessed for every feed due to the varying levels between mothers. This would be time consuming. Whilst standard blood analysis can be performed locally, a detailed assessment (examination of trace elements) would probably require testing at a regional level. This would result in further expense and could include organization between different local authorities. However, it would be beneficial in

assessing the affects of LCPUFA levels. Infants being fed EBM or formula with high levels of LCPUFAs could be compared to those fed low level diets.

The role of the external auditory meatus (EAM) in the characteristics of tympanometric data from the neonate population requires further work. The change in ear canal diameter has been examined, and this study would suggest that its interaction is not a major problem. However, the vibratory motion of the EAM is an important factor that needs to be properly understood. Measurements specifically on the EAM tissues would allow for quantification of this effect. The unusual morphology of the neonate tympanometric data needs to be fully understood before clinical application can be addressed. It would, thus, be of interest to test the ear canal with the tympanic membrane (TM) fixed. Alternatively, testing could be performed with the ear canal artificially stiffened[k]. This would create adult-like ear canal properties.

9.2 Future Work

More research should be performed on the evolution of normal neural and auditory function. It is thought that the continuing improvements in function are linked to nutrition. The role of parental nutrition is also an important consideration when investigating the maturational characteristics in the newborn. Essential fatty acid (EFA) levels in the mother are affected by pregnancy history, these levels reducing with each successive birth. Other areas affecting newborn health are genetic changes in the gene pool and alterations in female developmental characteristics. There is also a reported reduction in gender differences during the preterm period in recent times. A retrospective analysis would be of interest.

The possibility of a successive run effect for ABR testing should be studied. A single test was performed on the ear that was not selected for the study. This was used to validate results of the test ear. There was a tendency for this non-test ear to display poorer waveform morphology and prolonged latency data. Considering the test ear was chosen at random, this behaviour is not due to pathological problems. This aspect should be considered with further ABR testing that requires many test runs.

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[k] Collodion has previous been used to artificially stiffen the tympanic membrane. It could also be used on the ear canal wall.

The effects of incubator noise were not investigated during this study. The interaction of incubator noise (and tube feeding that could possible affect ME function) on the preterm auditory system would be an area of research to consider. The incubator noise (for enclosed style incubators) has been reported to be of a high level and is constant. This is not ideal for the immature auditory system of the preterm infant. To investigate the effects of the incubated environment, testing of both ABR and tympanometry would need to be undertaken. The testing limitations for this study did not allow for analysis of this aspect.

A model of the infant TM was established (presented in Appendix F). However, a detailed development and investigation of the model was not possible. A more detailed review of the structural and tissue properties of the TM would be necessary for a more sophisticated analysis to be performed. Data does exist on the mechanical properties of the TM and other tissues (particularly in the preterm population). However, it would be advisable to embark on this area of research in conjunction with a pathology specialist. This would allow testing for the mechanical properties of tissues specifically in the preterm infant. The full middle ear could be modelled in a similar manner.

In order for multi-frequency multi-component tympanometry to be of clinical use a greater number of term infants need to be tested. This would allow for a comprehensive normative dataset to be established. Case studies on abnormal subjects would allow the identification of response characteristics with differing pathologies. These case studies could be used to establish normative numerical data for clinical use. A greater understanding of pathologies and transient conditions in the term population would allow greater investigation and understanding of the more complex preterm tympanometric data.

There has been much research into otoacoustic emissions (OAE) in the newborn populations. However, there still exists a lack of data on the link between the different methods of auditory testing. A greater understanding on the links between tympanometry, OAEs and the ABR would be beneficial for the enhancement of clinical understanding and the progression of multi-method testing. The links between tympanometric data collected on this study and the ABR results were investigated in this thesis. Standardization of testing methods needs to be acquired for these different methods. This would aid comparative studies to be utilized for determining equivalent

testing methods. This could lead to reference methods being established that would provide reliable normative data for comparison between studies and testing methods.

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CHAPTER TWO - Auditory Brainstem Response (ABR)

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APPENDIX A

Original Study Protocol

This is the original study protocol as proposed by Hillingdon Hospital.

Appropriately grown term babies are born with high levels of Arachidonic (AA) and Docosahexaenoic (DHA) acids due to placental enrichment. This placental supply is denied if the mother has impaired fatty acid metabolism, placental infarction or the baby is delivered prematurely. It is known that preterm and low birthweight term babies are born with significantly reduced amounts of AA and DHA.

For maturity and integrity of AA and DHA rich structural membranes of the neurovascular, auditory and visual systems, a balanced provision of AA and DHA is required. A deficiency of these nutrients during any of the developmental stages would be manifested in clinical or sub-clinical disorders. It is now established that preterm babies fed on formula milk devoid of DHA during a brief window of the postnatal period have lower visual and stereo acuity, and cognitive ability.

Due to the high degree of unsaturation of AA and DHA, membranes rich in these two nutrients must be protected from peroxidation. Protection is provided primarily by the antioxidant enzymes and vitamins. There is interdependency between membrane-bound antioxidant enzymes, and AA and DHA; optimum activity of these enzymes is dependent on the physical integrity and maturation of the membranes, and the membrane AA and DHA require the enzymes for protection.

Our previous study has established that preterm and intra-uterine growth retarded babies have low concentrations of the antioxidant vitamins A and E, and depressed activities of the antioxidant enzymes Cu/Zn - superoxide dismutase, Se - glutathione peroxidase and Fe - catalase. The findings imply that lipid-rich membranes in preterm babies could be susceptible to peroxidation under an unfavourable environment.

This study proposes to investigate the role of AA, DHA and antioxidants in the maturation of the auditory system.

<u>SUBJECT GROUPS</u> (Approximately 40 - 50 babies per group)

GROUP 1 Preterm babies (<32 weeks gestation) or less than 1500g. Fed >75% volume of preterm milk formulation containing AA and DHA.

GROUP 2 Preterm babies (<32 weeks gestation) or less than 1500g. Fed >75% volume of breast milk.

GROUP 3 Preterm babies (<32 weeks gestation) or less than 1500g. Fed >75% volume of preterm milk formulation containing no AA or DHA.

GROUP 4 Term babies (3.5-4kg) - Control normative data.

Blood will be collected in heparinised tubes from each baby at birth (cord blood), and every two weeks until the expected date of delivery (40 weeks post conceptional age (PCA)). Blood will also be taken from the mothers at the time of delivery. Blood samples from mothers collected before the onset of labour, during labour and soon after delivery show no difference in fatty acid composition.

The red cells and plasma will be promptly separated by centrifugation. After washing the red cells with saline, both fraction will be flushed with nitrogen (if possible)

and stored at low temperature (-20°C). The saline used for washing the red cells needs to be discarded.

Parameters to be Assayed

Plasma - Vitamin A and E, zinc, copper and iron.
Red cells - Fatty acids; catalase, gluthathione peroxidase and superoxide dismutase.

Information Required

1. Record of pregnancy history of the mother, and term assessment of the infant.
2. Detailed record of the type and quantity of feed given to each baby during the duration of the study.
3. Number of transfusions (if any), amount transfused, time interval between each transfusion and sample of the transfused blood.
4. Data of developmental measurements of each infant.
5. Clinical details relevant to the investigation.
6. Packed cell volume (PCV) of all blood samples.

Procedure for collecting Blood Samples

1. Preterm babies under 32 weeks or 1.5kg at birth must have cord blood (5ml) taken at birth or soon after into heparinised tubes. Delivery rooms to have sample bottles (Orange heparin) available.
2. Parents to be asked after the birth to sign form of consent and be given information on the trial.
3. Nursery sisters on SCBU to inform Newborn Hearing Unit on new preterm arrivals for randomization of oral feeding.
4. All new preterms will be designated a number and a feed when the paediatricians feel this is indicated.
5. The first Monday morning after delivery, blood (1ml) will be taken and put into the Orange Heparinised bottles, which must be labelled with the baby's name and number. These bottles will go with the other samples to the Haematology Department within the hour. These bottles will have a blue spot on them to designate Hearing Research. They will come in their own plastic bags.
6. The specimens will be centrifuged and the red blood cells (RBC) and plasma separated and stored at -20°C in the deep freeze by the Haematology staff.
7. Brainstem traces will be carried out on a Monday and Tuesday morning on the preterm babies.

APPENDIX B

Mathematical Concepts of Acoustic Immittance

The Mechanical Analogy

The impedance concept is widely used in describing the properties of acoustical, mechanical and electrical systems. Impedance (Z) is defined as the total opposition offered by a system to the flow of energy. For a mechanical system

$$Z_m(ohm) = \frac{F}{V}(Ns/m) \text{.....(1)}$$

with force (F) and velocity (V). This is fine for static forces, since sound energy is sinusoidal in nature we must examine a mechanical system where the force varies with time (t). With a sinusoidal force of frequency (f) and amplitude (A), the force can be expressed as

$$F(t) = A\sin(2\pi f t) \text{.....(2)}$$

The resulting velocity caused by a sinusoidal force in a mechanical system will also be sinusoidal, this is assuming that the system (the ear) is linear.

The three types of mechanical elements being considered here are spring, mass and friction.

A spring offers opposition to movement, where the force is of a sinusoidal form, the impedance is characterized by a unique phase relation between force and the resulting velocity. With force lagging velocity by 90°, when force is maximum, the spring is maximally compressed with a zero velocity. Similarly, when the force is zero (midway between pushing and pulling), the spring is expanding and the velocity is at a maximum. The opposition offer by a spring to the flow of energy is its compliant reactance (X_c). Compliance (C) is the reciprocal of spring stiffness (K). Compliant reactance (X_c) can be expressed as

$$-X_c = \frac{1}{2\pi f C} \text{.....(3)}$$

The negative sign in this equation indicates the 90° phase lag of the force relative to the velocity response.

The mass (m) element in a mechanical system also has a unique relationship between sinusoidal force and the resulting velocity, the force will lead the velocity by 90°. In a frictionless environment, once the mass is set into motion it will remain in motion until a force stops it, this is due to the inertia of a mass. An applied force will set the mass into motion in the direction of the force, when the force reaches zero the velocity will be maximal, a force in the opposite direction will bring the motion to a halt. Correspondingly, when the force is at a maximum, the velocity will be zero. The opposition offered by a mass to the energy flow is its mass reactance (X_m). Mass reactance (X_m) can be expressed as

$$X_m = 2\pi f M \text{.....(4)}$$

The third element is friction, the applied force and resulting velocity are in phase. This frictional opposition is termed resistance (R). Resistance differs from the reactance elements in that resistive elements dissipate energy where as reactive elements store energy. Ideal reactive elements therefore do not lose energy, the resistive elements on the other hand lose energy by converting it to heat.

Impedance of Complex Mechanical Systems

As previously mentioned, a system such as the ear is a complex system consisting of all three processes. Considering initially the masses involved, in an ideal mechanical system (assuming no friction) consisting of two mass elements in series, the total impedance will be the simple sum of the two mass reactances. However, complex systems are rarely this simple, if we consider a mechanical system consisting of three different elements in series; a mass, a spring, and a resistance. For a sinusoidal force, the velocity response of the mass will be exactly opposite (180° out of phase). Therefore, the total reactance (X_t) of a system consisting of a mass and a spring in series will be the algebraic sum of the mass reactance (X_m) and the compliant reactance (X_c).

$$X_t = X_m - X_c \text{.....(5)}$$

The system is said to be at resonance when the absolute values of mass and compliant reactance are equal. The system can also be characterized as mass or compliance (equivalently stiffness) controlled when either value is larger than the other.

In order to establish the impedance, the resistance of the system must be determined. At this point, it beneficial to introduce the vector system for defining impedance (and admittance) components. By convention, resistance is represented as a vector on the x-axis, the vector length representing the resistance amplitude. The resistance vector phase angle being 0° due to the in-phase force-velocity relationship characterizing resistance. Mass reactance (X_m) is represented as a vector projecting in the positive y-axis direction, phase angle of +90° due to the phase lead (force relative to velocity). Finally, compliant reactance (X_c) is represented as a vector in the negative y-axis direction due to the -90° phase angle. The total reactance (X_t) is therefore the difference in length of the X_m and X_c vectors. Due to the similarity of the vector plot to the conventional representation of complex numbers, resistance is referred to as the real component, reactance being the imaginary component. The equivalent vector plot is used for the components of admittance; conductance being real and susceptance imaginary.

The total impedance of a system is given by the vector sum of resistance (R) and the total reactance (X_t). In order to express the impedance completely, the magnitude value of the vector must be accompanied by the phase angle. The magnitude is calculated using the Pythagorean theorem

$$|Z| = \sqrt{R^2 + X_t^2} \text{.....(6)}$$

The angle is determined by the relative values of R and X_t, θ being measured from the real axis (an anti-clockwise direction denoting a positive θ value).

$$\tan\theta = \frac{X_t}{R} \text{.....(7)}$$

The phase angle represents the temporal relationship between the velocity of the complex system and the sinusoidally applied force.

Admittance of Complex Mechanical Systems

It is sometimes more convenient to measure admittance, this is defined as the ease with which energy flows into a system. Mechanical admittance (Y_m) is mathematically expressed as

$$Y_m = \frac{V}{F} \text{.....(8)}$$

with Y_m measured in mho's

$$Y_m(mho) = m/Ns \text{.....(9)}$$

It can be seen from equations (1) and (8) that admittance is the simple reciprocal of impedance, hence

$$|Y| = \frac{1}{|Z|} \text{.....(10)}$$

Admittance has components equivalent to the resistive and reactive elements of impedance. Mass susceptance (B_m) is the ease of energy flow into a mass, compliant susceptance (B_c) is the ease of energy flow into a spring. Finally, conductance (G) is the ease with which energy flows into a resistive (frictional) element. The mathematical conversions from impedance to admittance are as follows

$$B = \frac{-X}{R^2 + X^2} \text{.....(11)}$$

$$G = \frac{R}{R^2 + X^2} \text{.....(12)}$$

Similarly,

$$X = \frac{-B}{G^2 + B^2} \text{.....(13)}$$

$$R = \frac{G}{G^2 + B^2} \text{.....(14)}$$

Derivations for equations (11) to (14) can be seen at the end of this appendix[A].

Acoustic Immittance of the Ear

Acoustic impedance is defined as the total opposition offered by a system to the flow of acoustic energy. Acoustic impedance Z_a can be measured by applying a sound pressure P, and measuring the resulting volume velocity U in the sound conducting medium. Mathematically, this can be expressed as

$$Z_a = \frac{P}{U} \text{.....(15)}$$

As sound is a time-varying pressure wave, sound pressure (P) is expressed as the root mean square (rms) pressure. The volume velocity, U, is measured as the rms volume that flows past an imaginary surface. Sound pressure is measured in N/m^2 (more commonly Pascals, Pa), and volume velocity in m^3/sec. Acoustic impedance is generally measured in ohms, where

$$Z_a(ohm) = 10^5\ Pa.\text{sec}/\ m^3 \text{.....(16)}$$

Since admittance is the reciprocal of impedance, acoustic admittance Y_a, is measured in mho (mmho due to typical values).

$$Y_a(mmho) = m^3 x 10^{-8}\ Pa.\text{sec} \text{.....(17)}$$

The acoustic impedance at the probe tip in the ear canal can be measured by presenting a constant volume velocity and measuring the resulting pressure. Alternatively, and with the equipment in use for this study, a constant sound pressure can be presented and a measurement of the resulting volume velocity will determine the acoustic admittance.

The acoustic immittance measured in the plane of the probe tip in the ear canal is a complex combination of both acoustical and mechanical elements of the ear. The acoustic immittance of the ear canal is heavily affected by the behaviour of mechanical structures in the ear. For example, the eardrum behaves like a mechanical spring, after displacement it tends to spring back to its neutral position. This has a similar effect as an acoustic compliance. In a similar fashion, the mechanical mass of the ossicles are reflected back to the plane of the probe tip, effecting the acoustic mass elements. The acoustic immittance is also affected by the resistance of the ear, mainly occurring in the cochlea. The ear, can thus, be represented as a complex mechanoacoustic system containing compliant, mass, and resistive elements.

The Effect of Ear Canal Volume

Acoustic immittance measurements are taken by inserting a probe into the external auditory meatus, the volume of air present in the ear canal has its own acoustic immittance and must be taken into account. This volume can vary considerably, being dependant on physical ear canal size, selection of probe tip and the depth to which the probe is inserted (volume can thus vary between tests). It is, therefore, necessary to assess the effect of ear canal volume for every immittance measurement.

The commercial approach in gaining compensated acoustic immittance measurements is to assume that the ear canal can be represented as an enclosed volume of air that is in parallel with the immittance of the middle ear. The input impedance of a parallel system is determined by

$$\frac{1}{Z_a} = \frac{1}{Z_{ec}} + \frac{1}{Z_{tm}} \quad \text{.....(18)}$$

Solving for Z_a

$$Z_a = \frac{Z_{ec}.Z_{tm}}{Z_{ec} + Z_{tm}} \quad \text{.....(19)}$$

Z_a is the acoustic impedance at the plane of the probe tip, Z_{ec} and Z_{tm} represent the impedances of the ear canal and the middle ear (at the plane of the TM) respectively.

Estimation of Ear Canal Immittance

In order to estimate the ear canal immittance, the ear canal volume must be determined. This can be done by measuring the volume of liquid (eg. alcohol) it takes to fill the ear canal, this however is not practical for routine clinical use. Conventional clinical procedure is to use tympanometry.

We can apply equation (18) to the acoustic impedance of the ear. By pressurizing the ear canal to a relatively extreme positive or negative value the impedance of the middle ear can be approximated to an infinitely high value. At high positive or negative pressure the eardrum is stiffened sufficiently that the ear canal can be acoustically modelled as a rigid walled cavity. Equation (18) will thus reduce to

$$\frac{1}{Z_a} = \frac{1}{Z_{ec}} \quad \text{.....(20)}$$

Taking reciprocals gives

$$Z_a = Z_{ec} \quad \text{.....(21)}$$

Correcting for Ear Canal Volume

The impedance of the middle ear can be found using equation (19). Solving equation (19) for Z_{tm} we obtain

$$Z_{tm} = \frac{Z_{ec}.Z_a}{Z_{ec} - Z_a} \quad \text{.....(22)}$$

Thus, using equation (22) we can estimate the impedance of the middle ear for any value of ear canal pressure. The corrected and uncorrected impedance tympanograms will

differ in morphology due to their non-linear relationship. Similarly, since admittance is the simple reciprocal of impedance, equation (18) can be written as

$$Y_a = Y_{ec} + Y_{tm} \text{ .....(23)}$$

It is often more convenient to measure admittance values due to the linear nature of the relationship. Solving equation (23) for middle ear admittance (Y_{tm}) gives

$$Y_{tm} = Y_a - Y_{ec} \text{ .....(24)}$$

Equation (24) simply states that the admittance values measured in the plane of the probe tip (Y_a) can be corrected to the plane of the middle ear (Y_{tm}) by subtraction of a constant (Y_{ec}), representing the admittance of the ear canal. The equipment used in this study provides admittance rather than impedance data.

Corrected and uncorrected admittance tympanograms are parallel in form due to the linear relationship of equation (24). There is an important assumption implicit to the use of these equations for the correction of impedance and admittance measurements. It is necessary to assume that the phase angle of the ear canal impedance is identical to the phase angle of the middle ear impedance. This assumption is fairly accurate for normal adult ears at low probe tone frequencies when the ear canal and middle ear are essentially compliant elements. This is not the case for many abnormal ears, for normal ears tested at higher probe tone frequencies, and for normal neonate ears even at low probe tone frequencies. In these cases, separate measurement of individual impedance components (resistance and reactance) or admittance components (conductance and susceptance) is required. The individual components can be corrected for ear canal volume separately, then impedance or admittance data formulated. For this reason, equipment used in the study will measure the real and imaginary components of admittance (conductance and susceptance) separately.

[A] ***Derivation of equations (11) to (14)***

The relations between the components of admittance with those of impedance are found by employing the algebra of complex numbers, where $j^2 = -1$. For the admittance quantity, expressed as a complex number, we get

$$Y = \frac{1}{Z} = \frac{1}{(R + jX)}$$

Multiplying the numerator and denominator of the above expression with the conjugate complex quantity R - jX, we get

$$Y = \frac{(R - jX)}{(R + jX)(R - jX)} = \frac{(R - jX)}{(R^2 + X^2)}$$

Identifying the real and imaginary parts of this expression with the ones of

$$Y = G + jB$$

This leads to the equations

$$G = \frac{R}{(R^2 + X^2)}$$

$$_{(J)}B = \frac{^{-}{}_{(J)}X}{(R^2 + X^2)}$$

Similarly, from

$$Z = \frac{1}{Y} = \frac{1}{(G + jB)} = \frac{(G - jB)}{(G^2 + B^2)}$$

Using

$$Z = R + jX$$

Leads to equations

$$R = \frac{G}{(G^2 + B^2)}$$

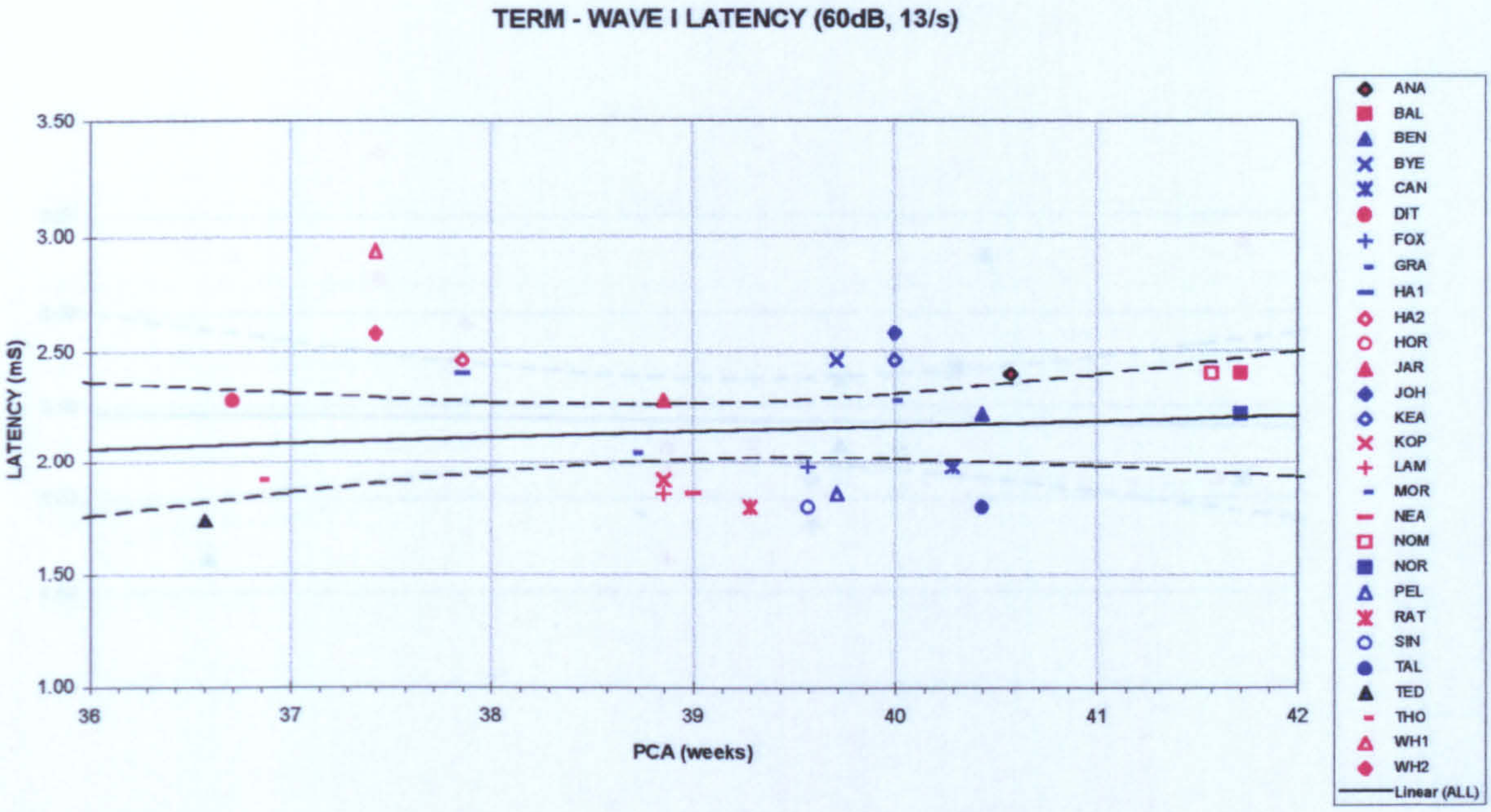
$$_{(J)}X = \frac{^{-}{}_{(J)}B}{(G^2 + B^2)}$$

Appendix C

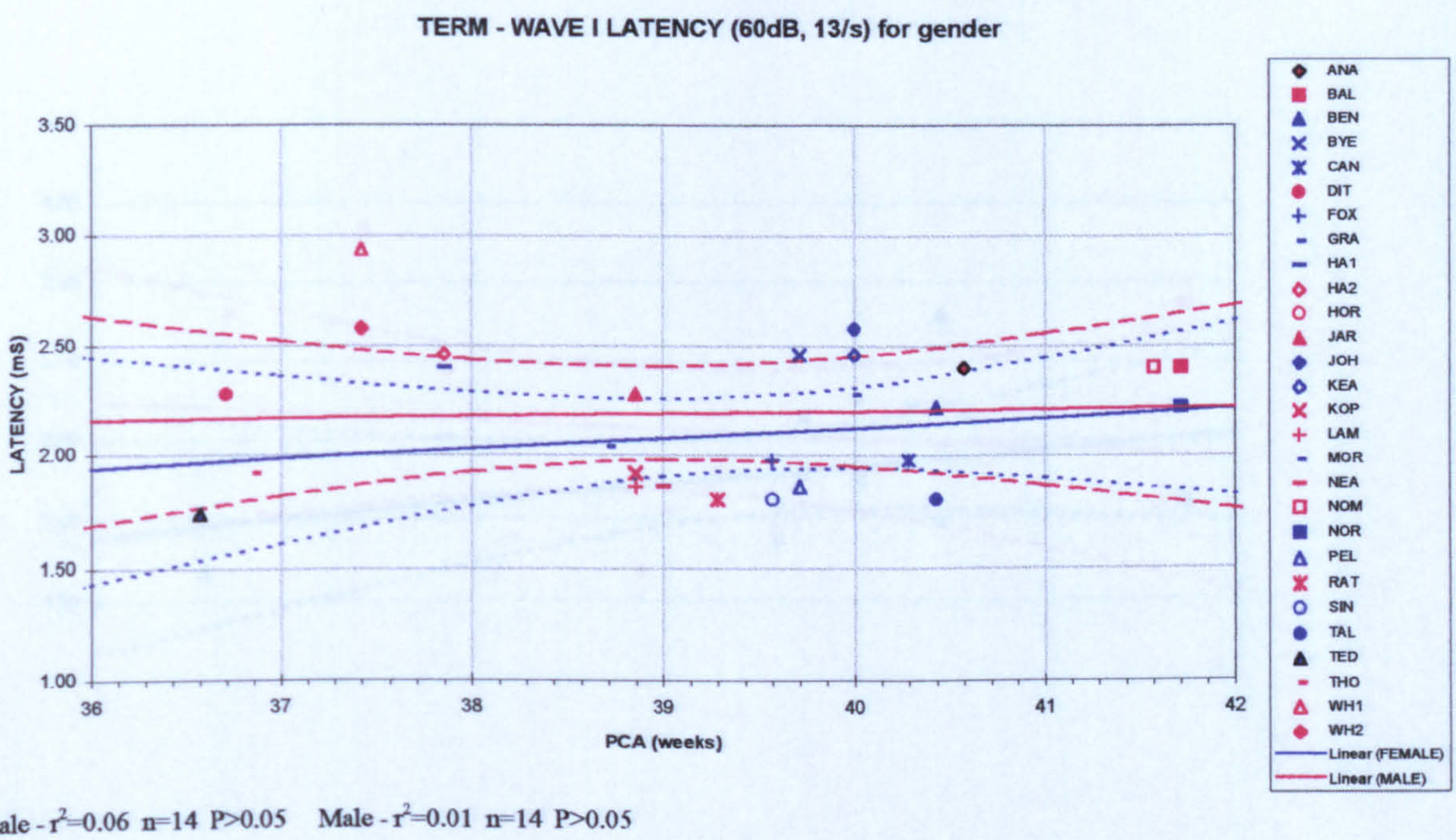
Term ABR study plots

This appendix contains raw data in the form of scatter plots from which all L-A functions are constructed. Confidence bands for the L-A functions at the 95% level are displayed. The Pearson correlation coefficient, subject numbers and significance levels for the one-sample t test are also included. Data is marked for gender (RED - Male, BLUE - Female) unless otherwise stated.

Figure C1 a/b

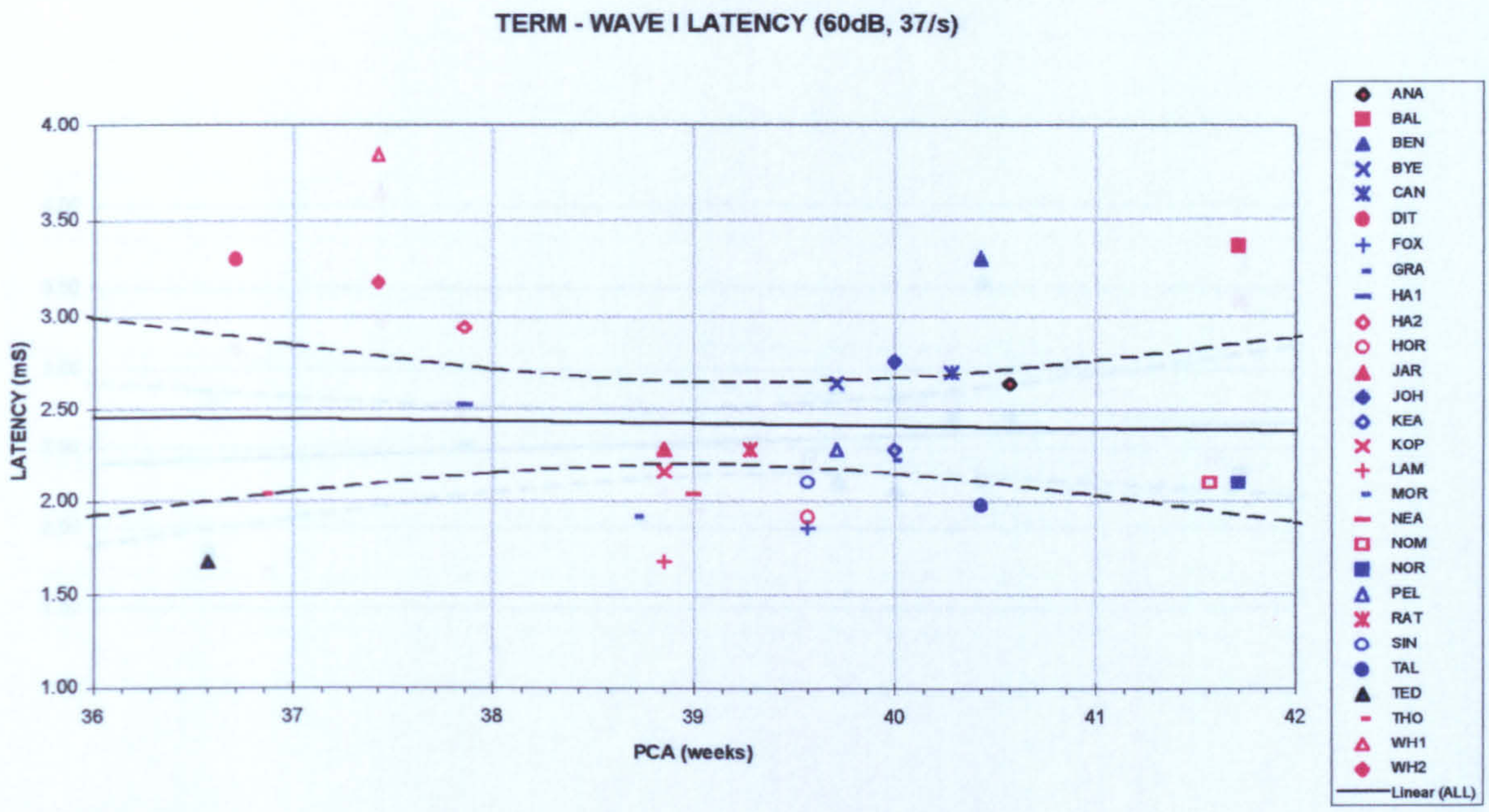


r^2=0.00 n=28 P>0.05

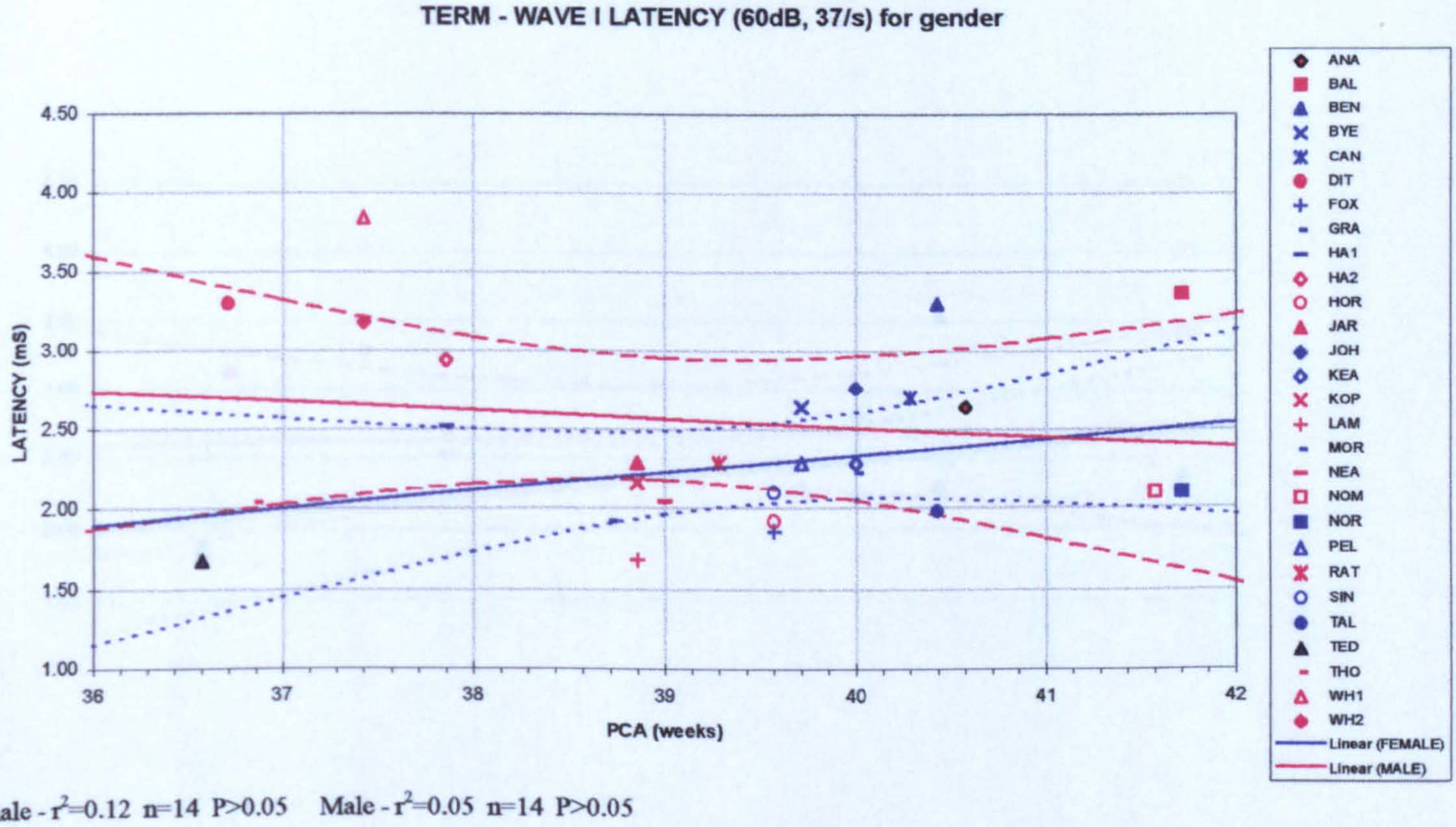


Female - r^2=0.06 n=14 P>0.05 Male - r^2=0.01 n=14 P>0.05

Figure C2 a/b

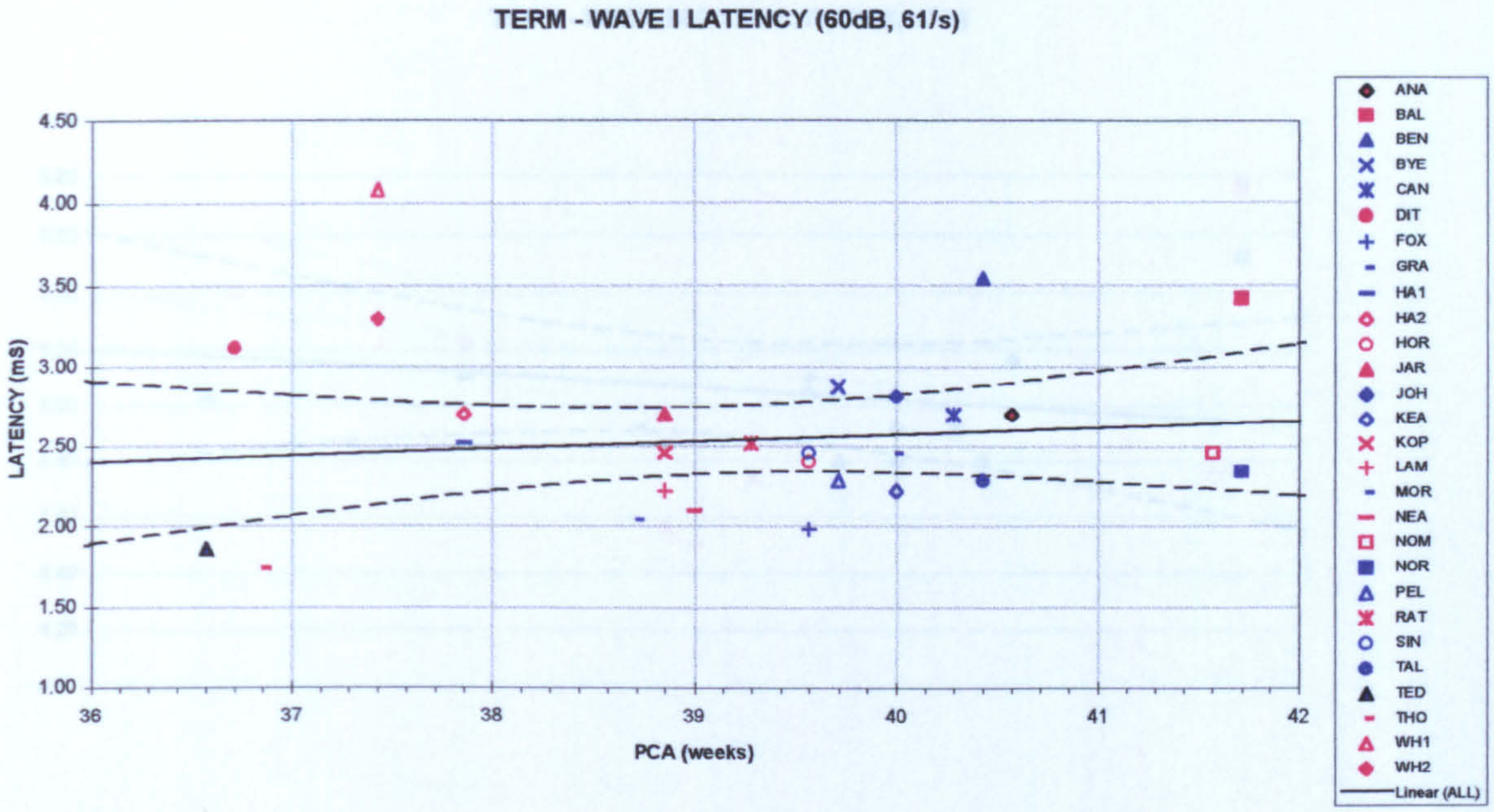


r^2=0.01 n=28 P>0.05

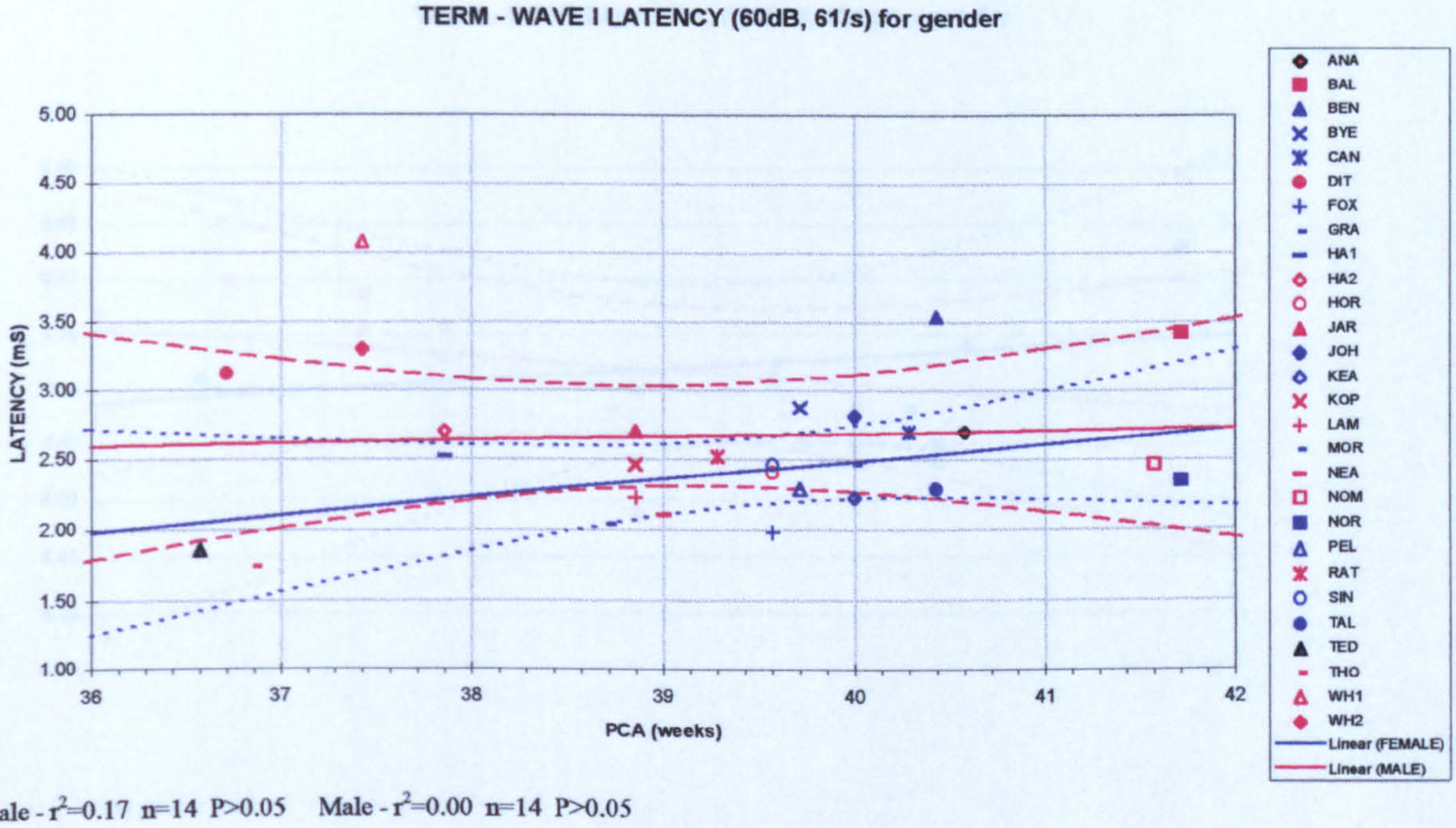


Female - r^2=0.12 n=14 P>0.05 Male - r^2=0.05 n=14 P>0.05

Figure C3 a/b

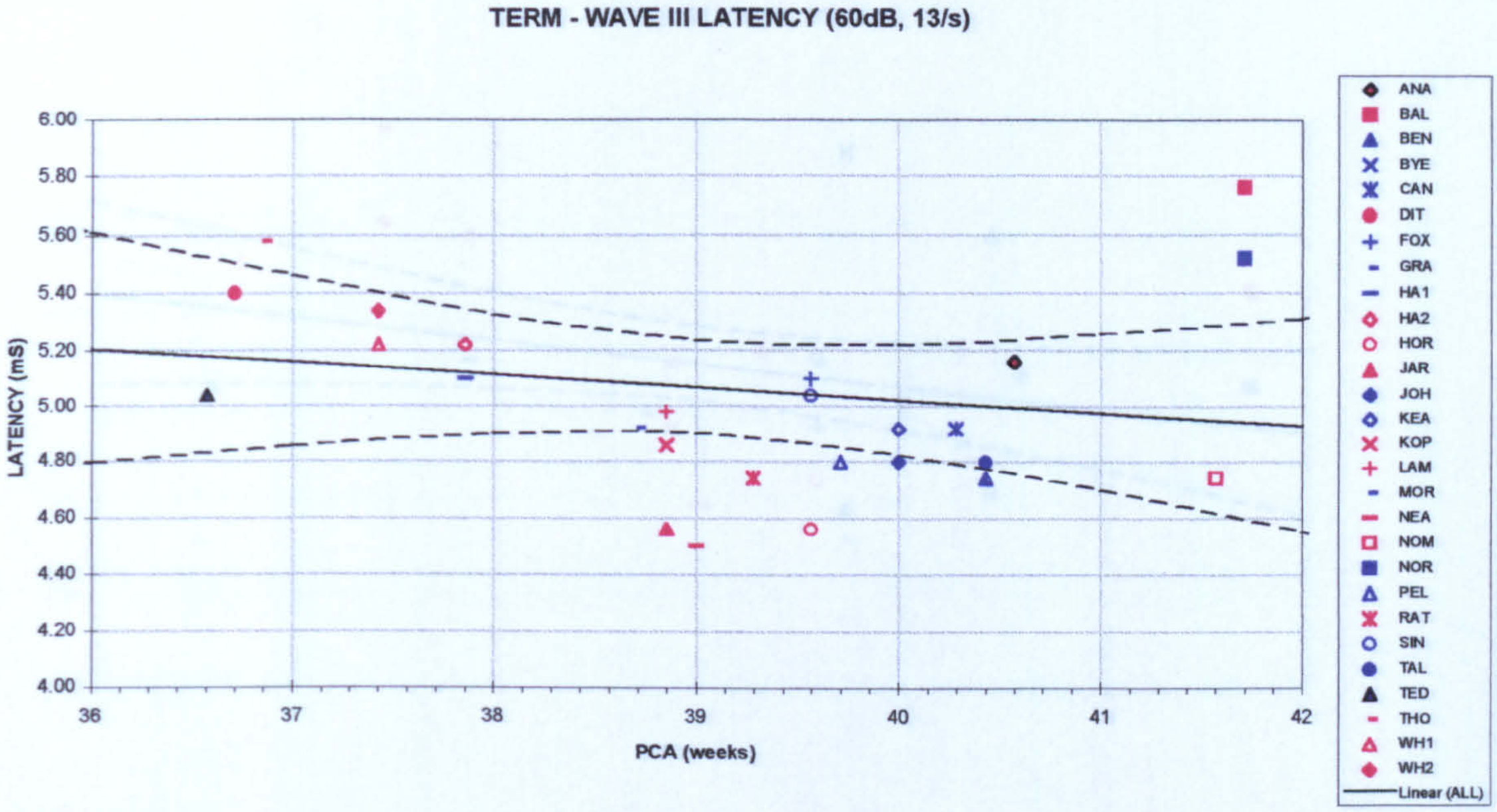


r^2=0.00 n=28 P>0.05

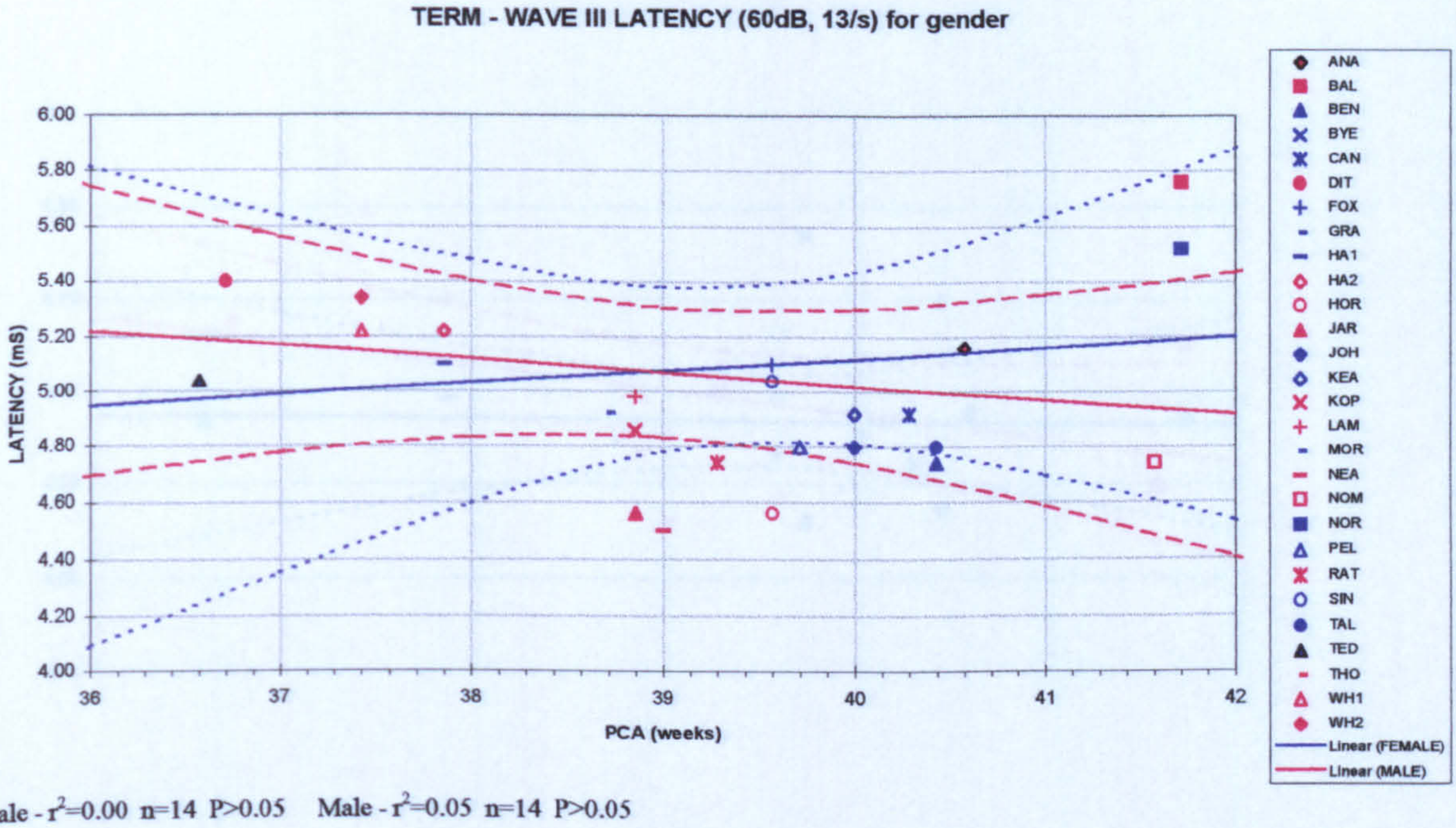


Female - r^2=0.17 n=14 P>0.05 Male - r^2=0.00 n=14 P>0.05

Figure C4 a/b

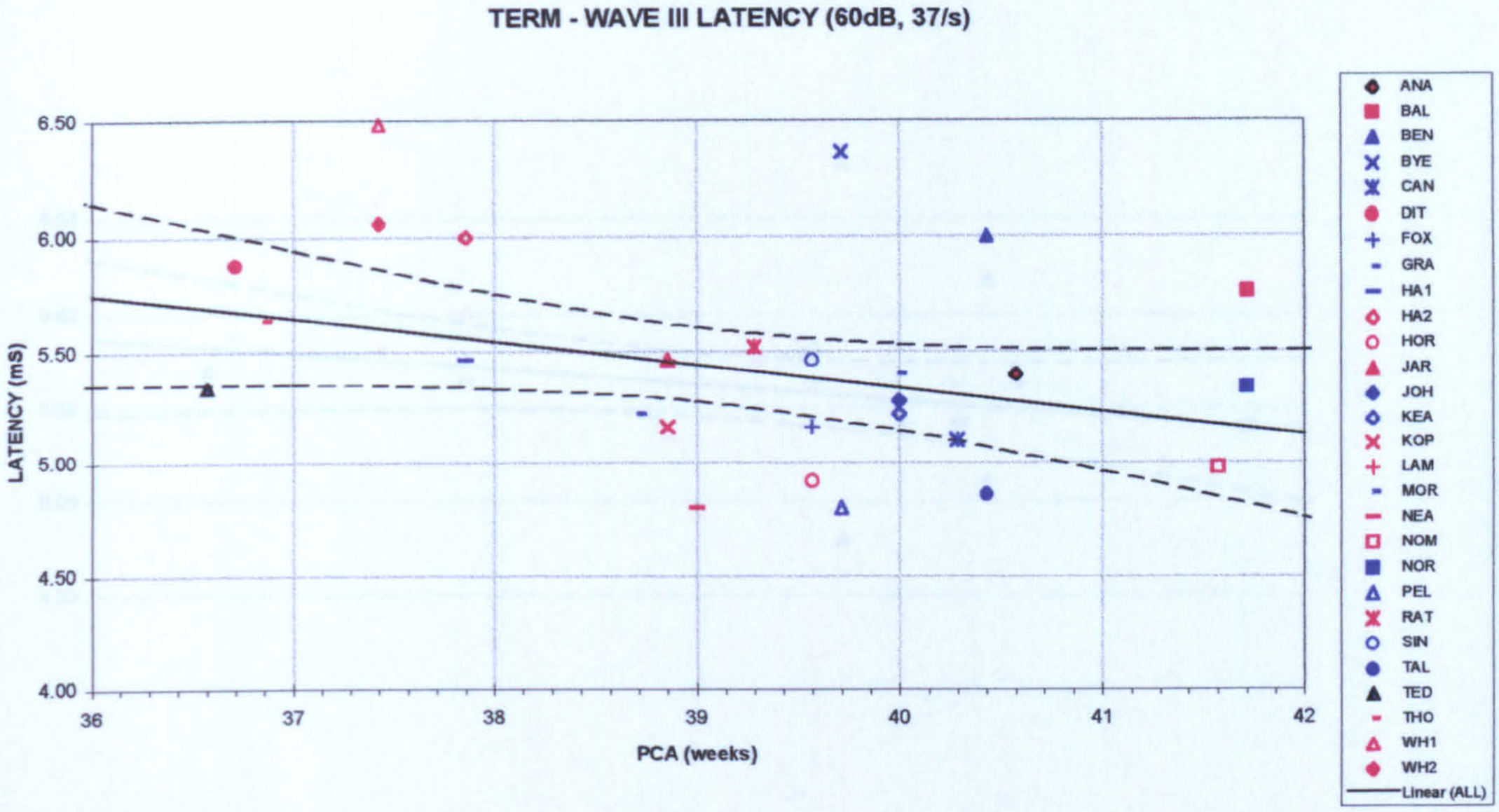


r^2=0.01 n=28 P>0.05

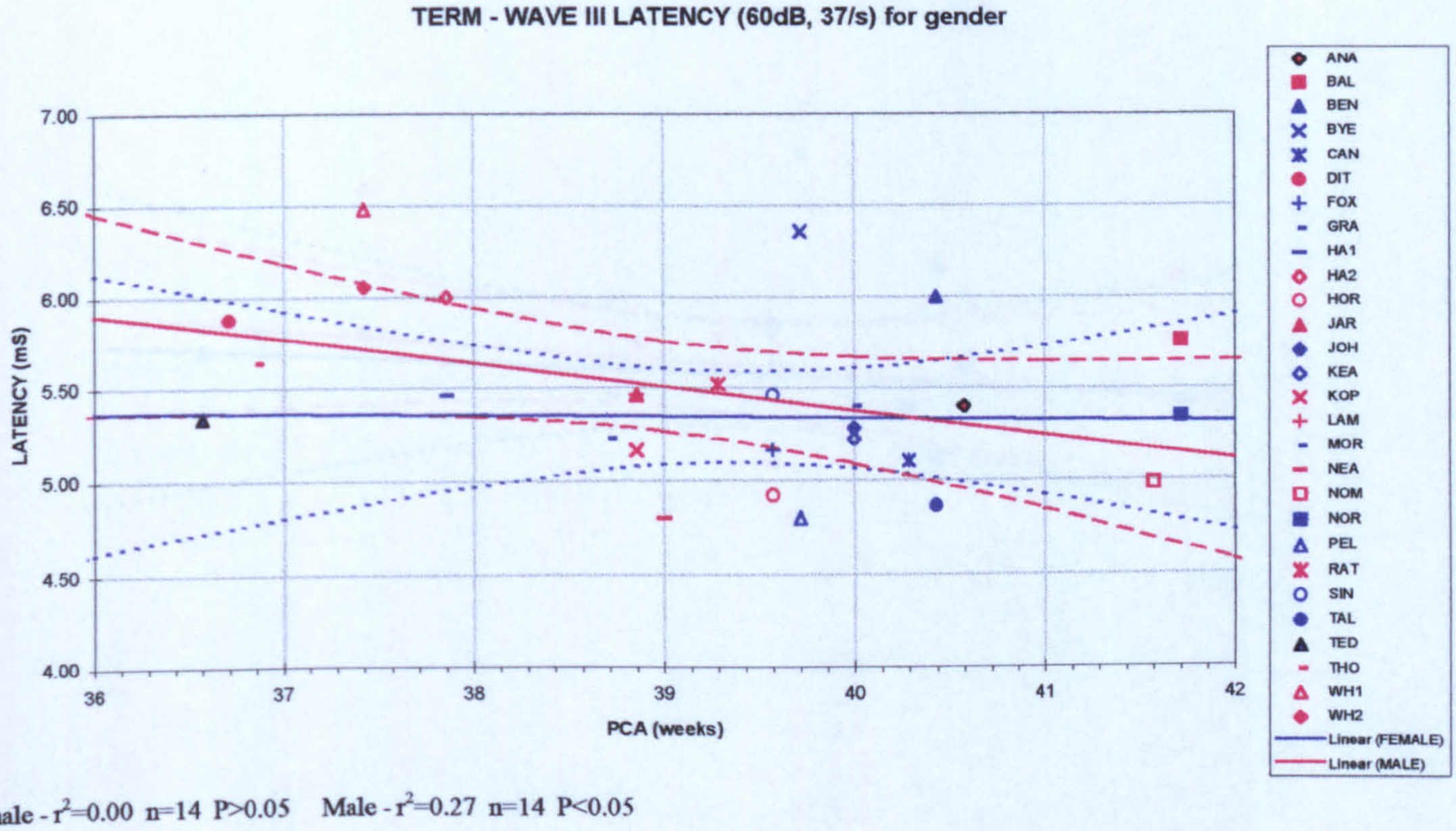


Female - r^2=0.00 n=14 P>0.05 Male - r^2=0.05 n=14 P>0.05

Figure C5 a/b

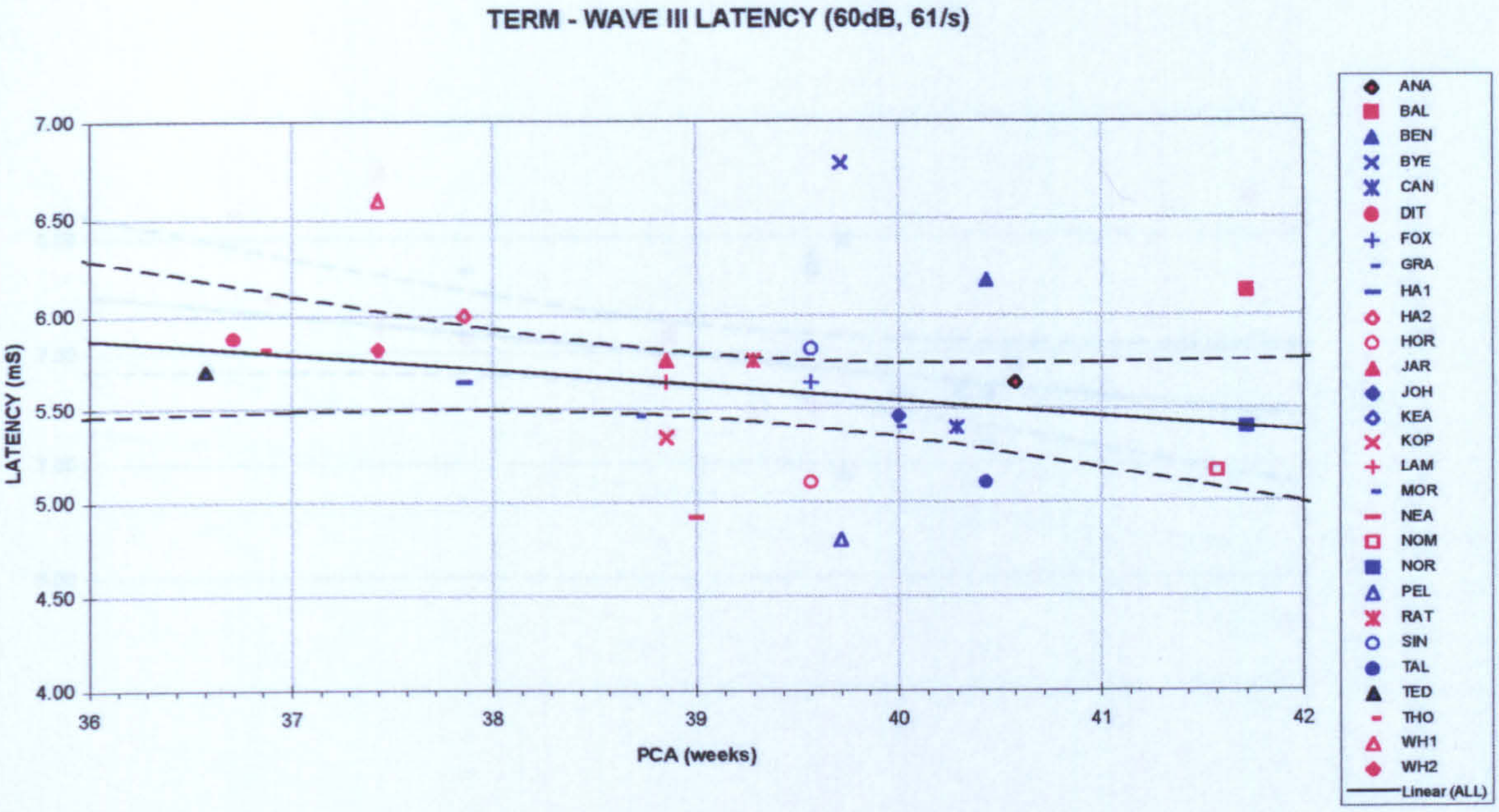


r^2=0.13 n=28 P>0.05

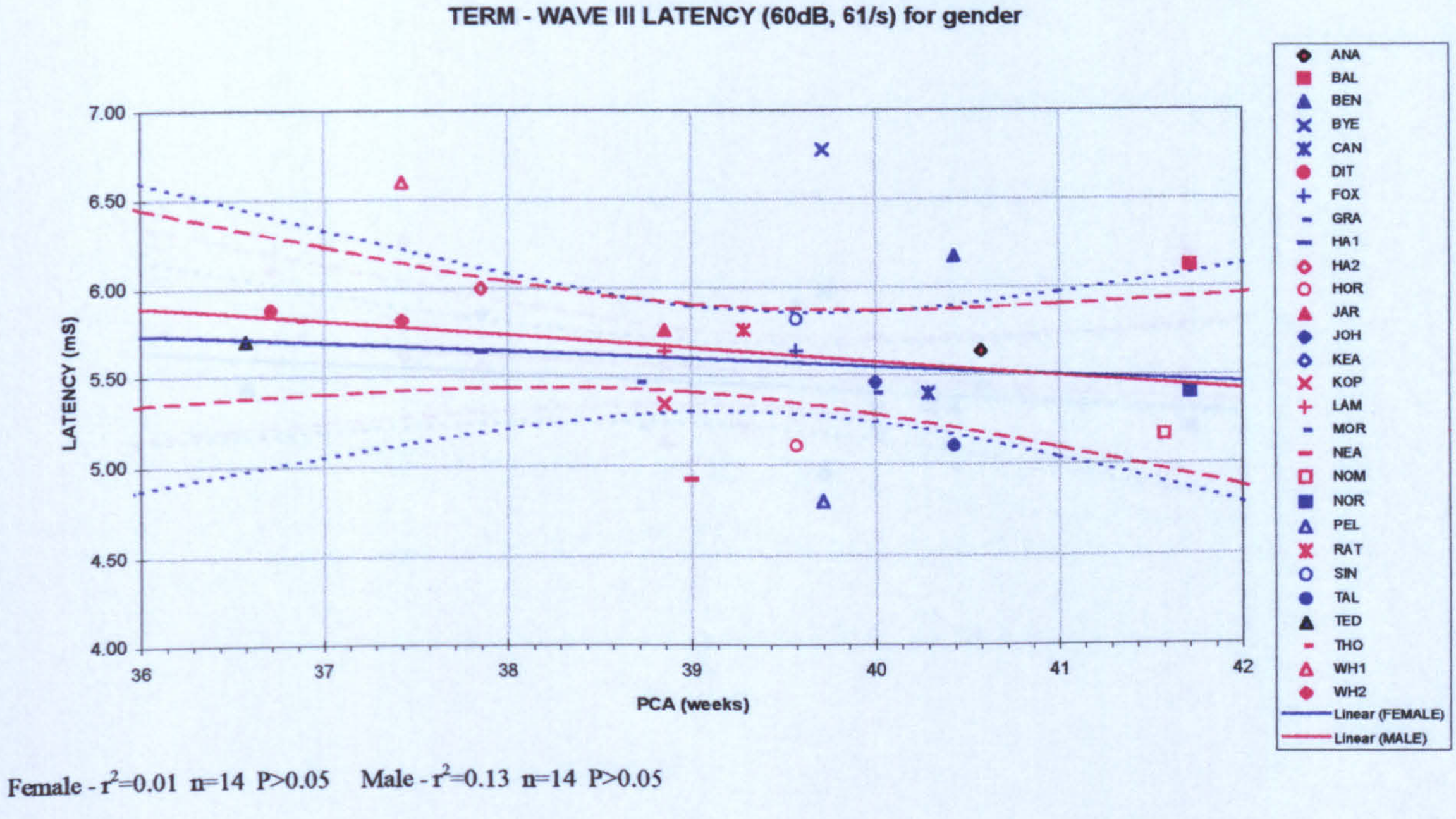


Female - r^2=0.00 n=14 P>0.05 Male - r^2=0.27 n=14 P<0.05

Figure C6 a/b

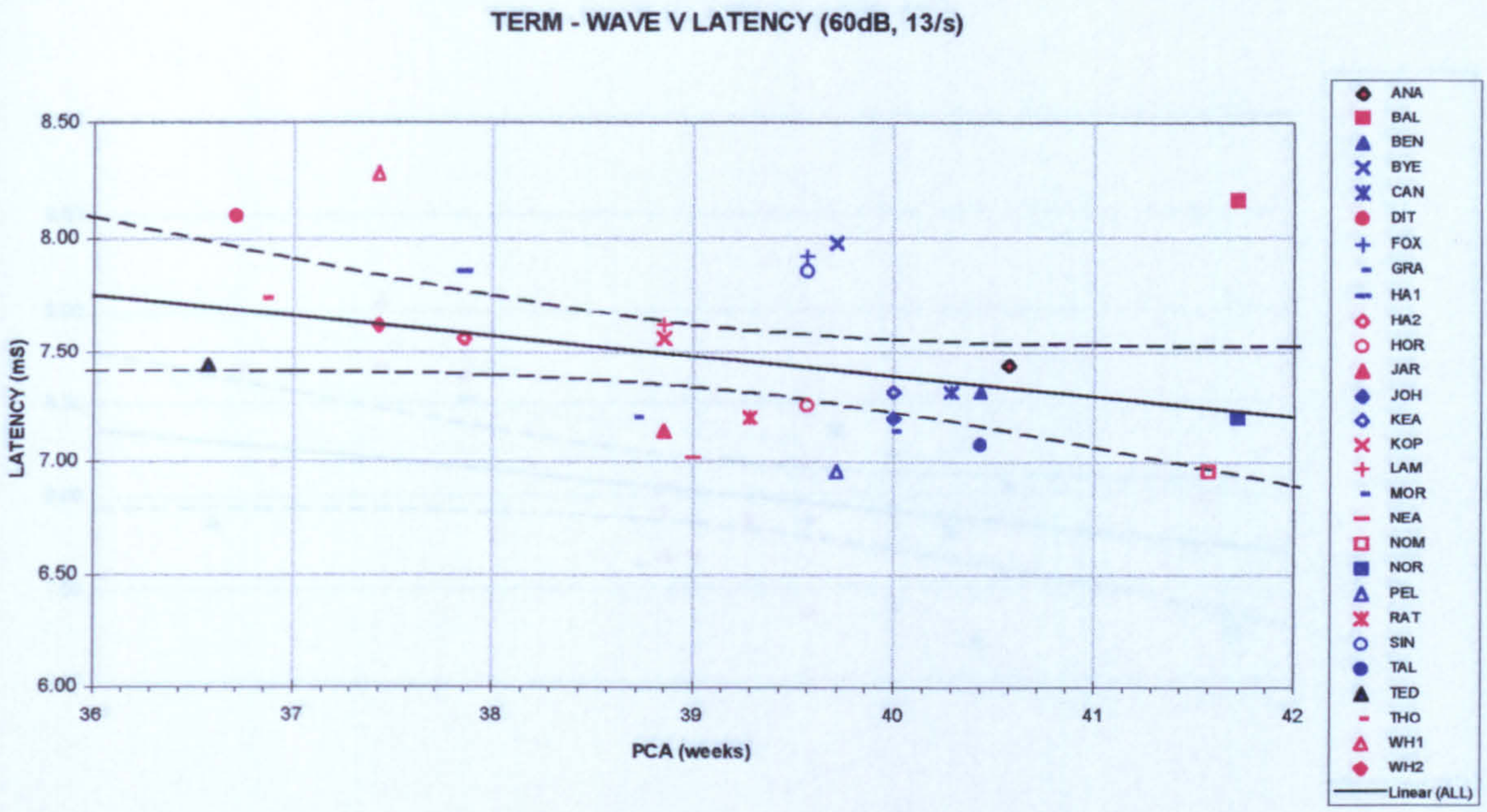


r^2=0.07 n=28 P>0.05

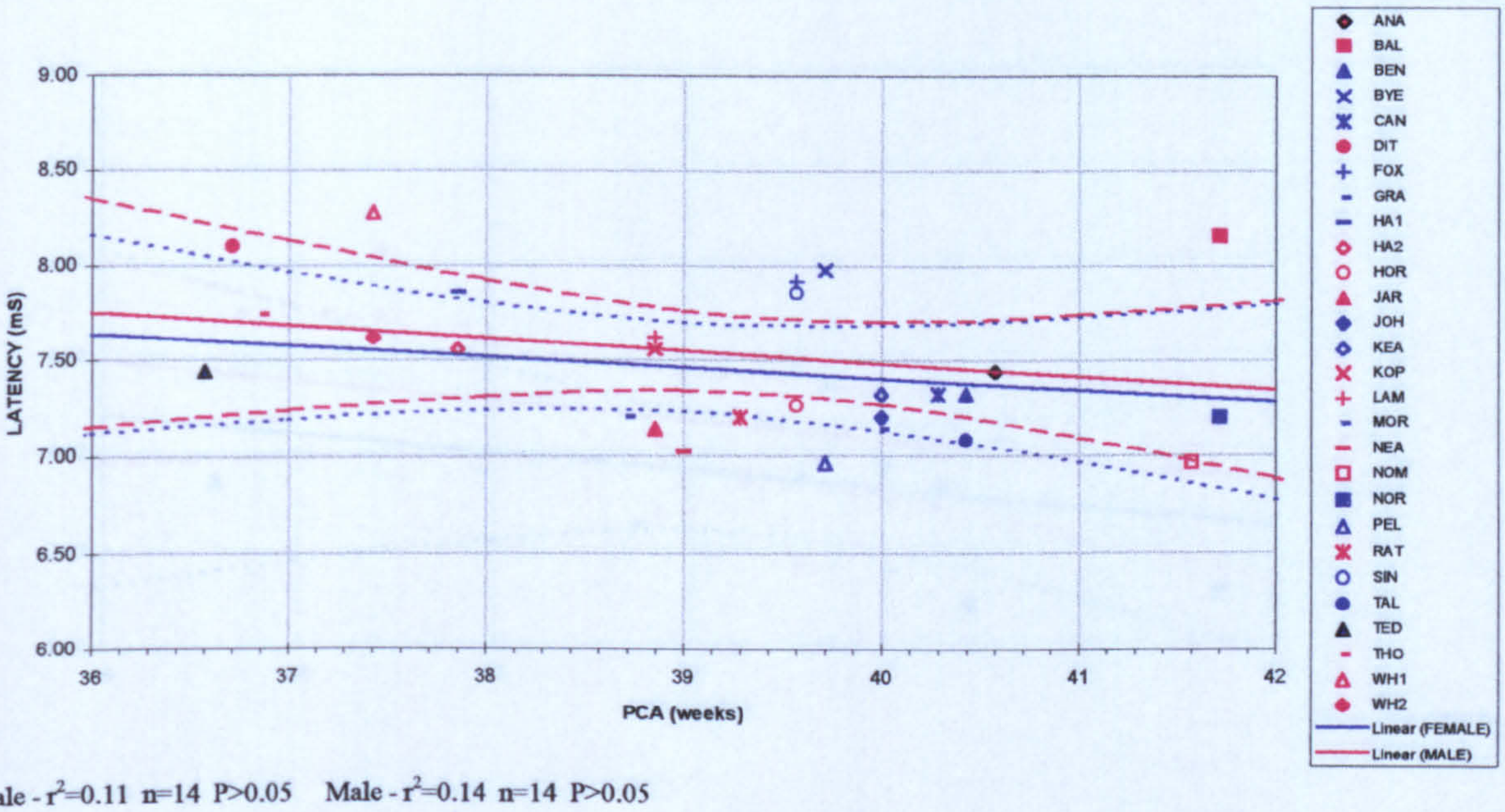


Female - r^2=0.01 n=14 P>0.05 Male - r^2=0.13 n=14 P>0.05

Figure C7 a/b

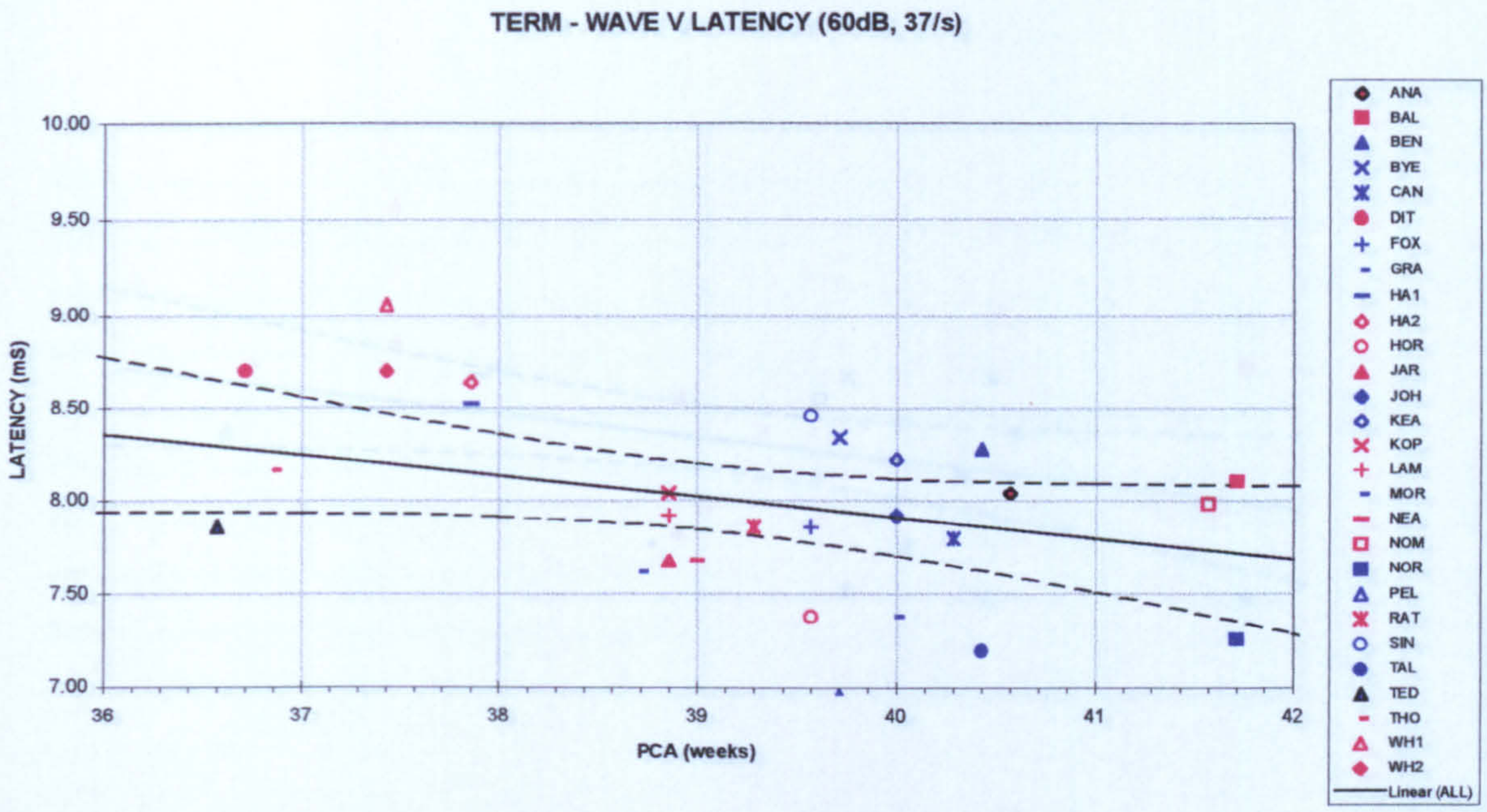


r^2=0.14 n=28 P>0.05

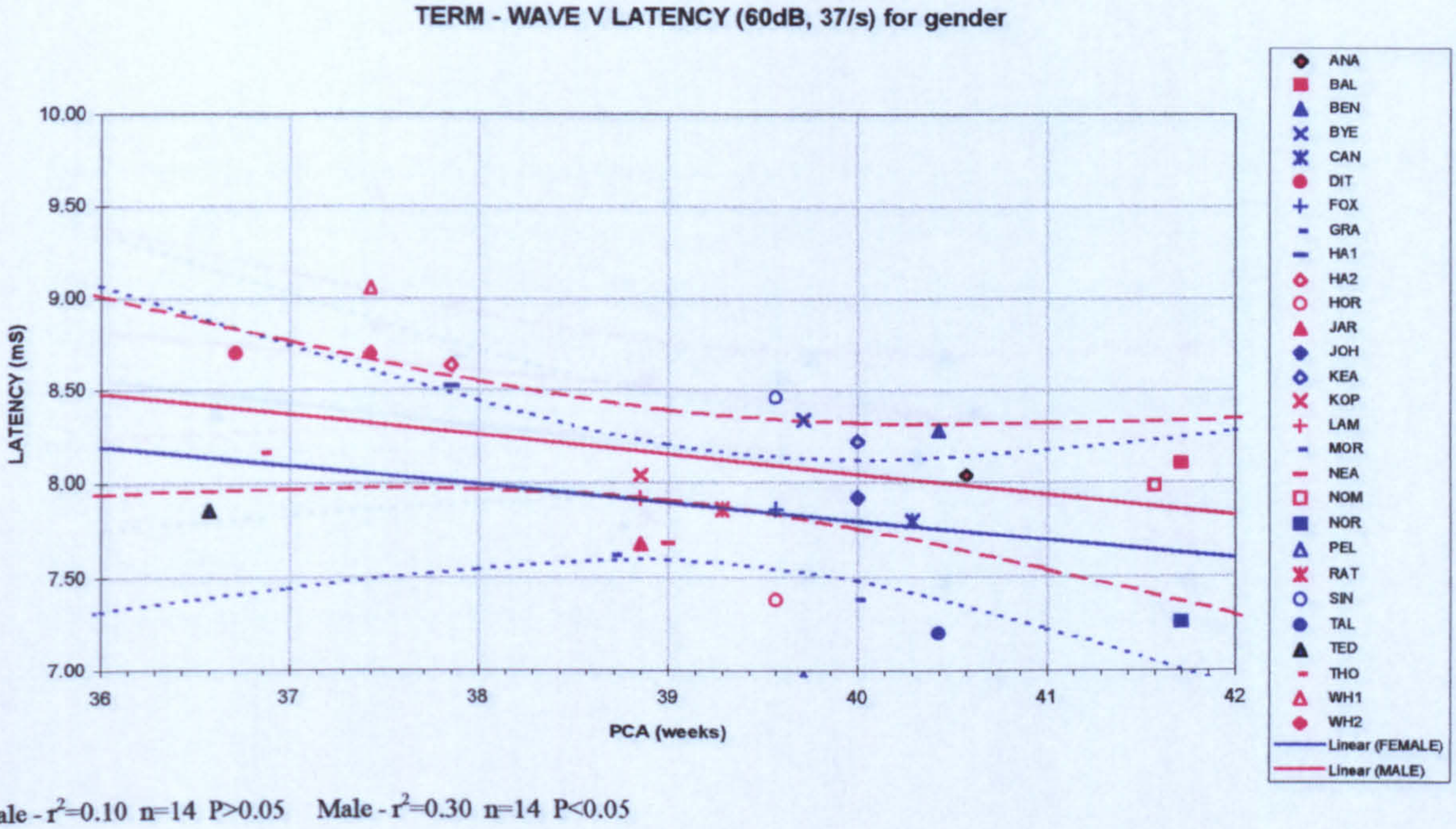


Female - r^2=0.11 n=14 P>0.05 Male - r^2=0.14 n=14 P>0.05

Figure C8 a/b

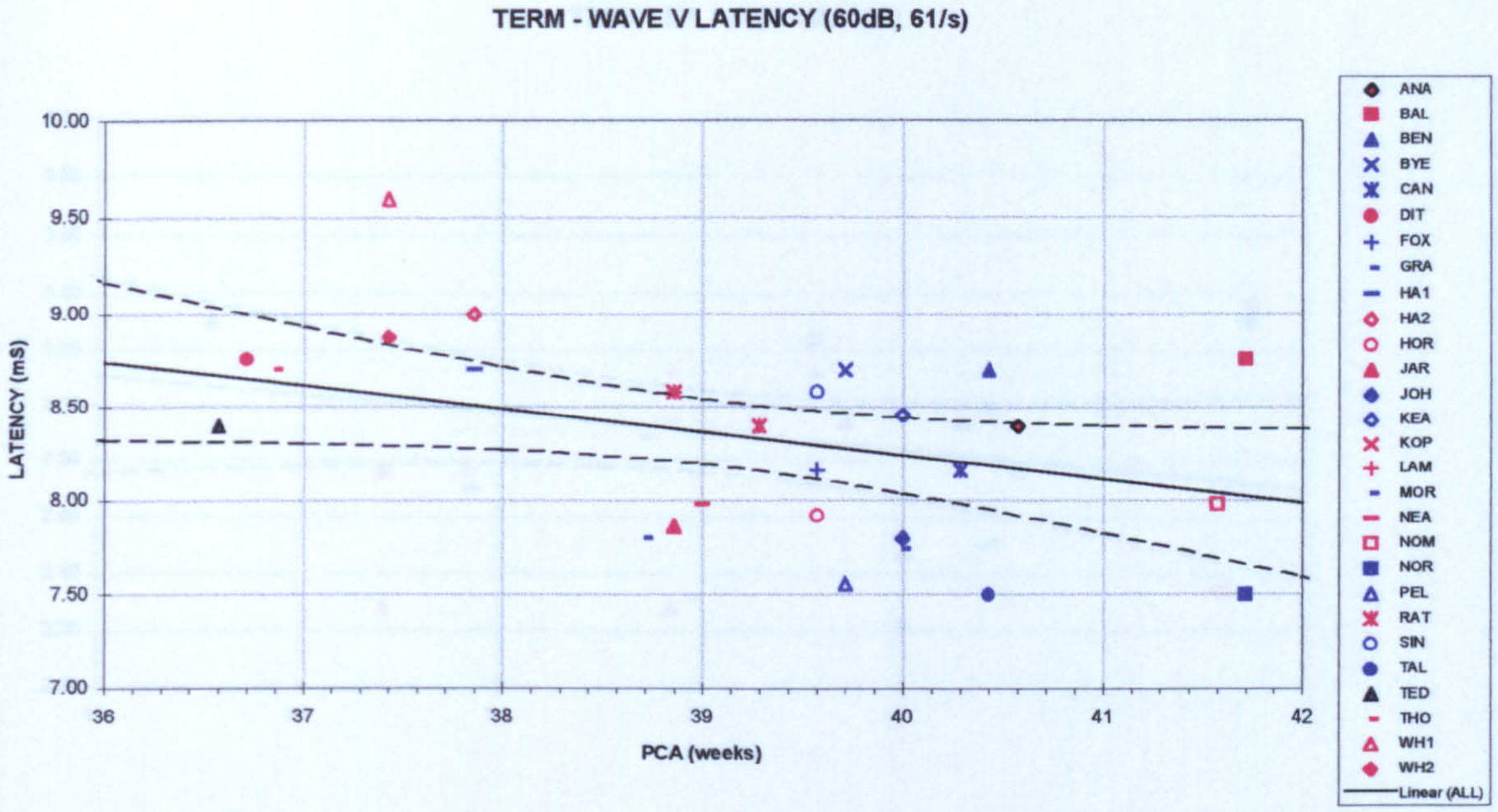


r^2=0.23 n=28 P<0.01

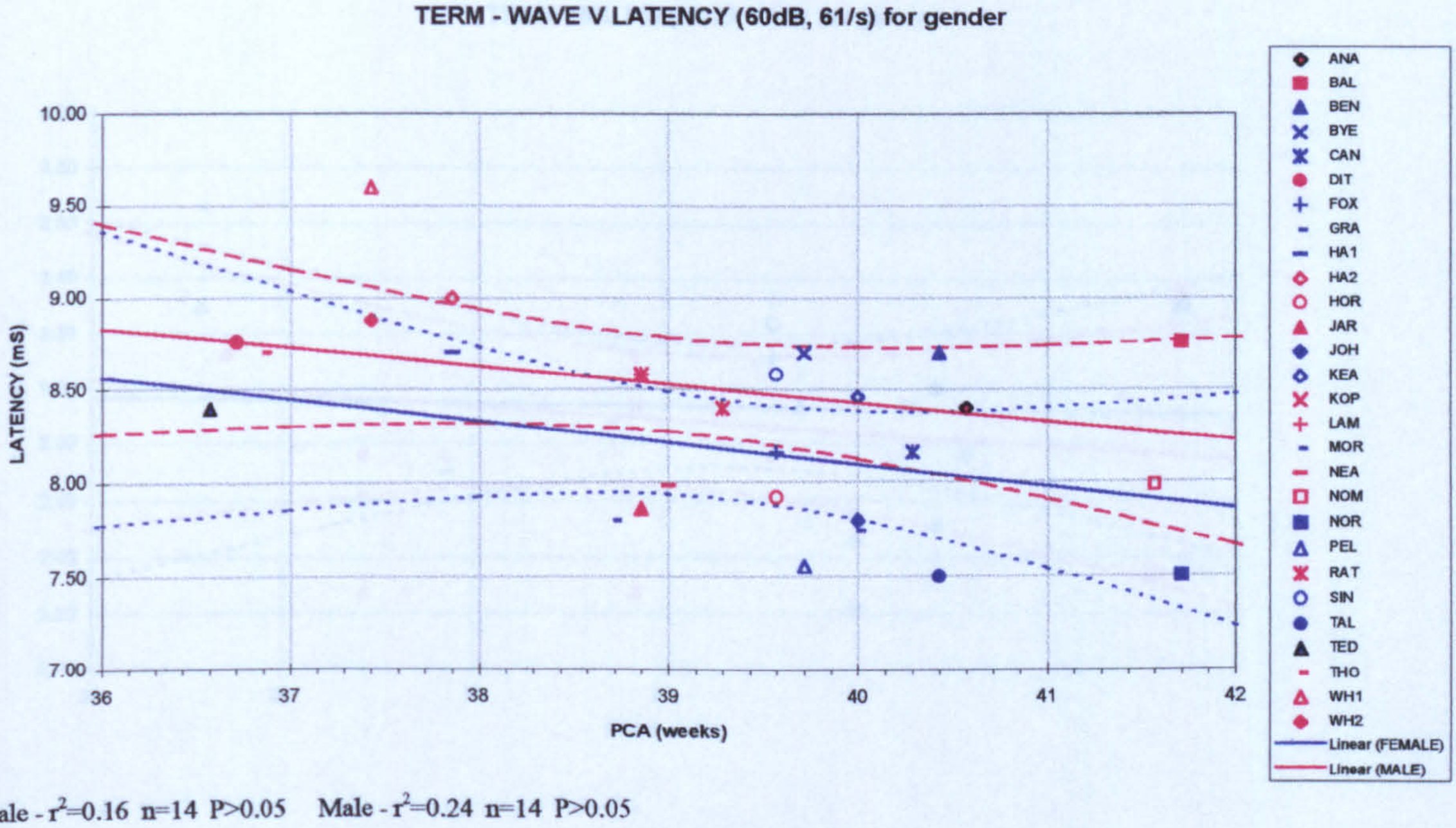


Female - r^2=0.10 n=14 P>0.05 Male - r^2=0.30 n=14 P<0.05

Figure C9 a/b

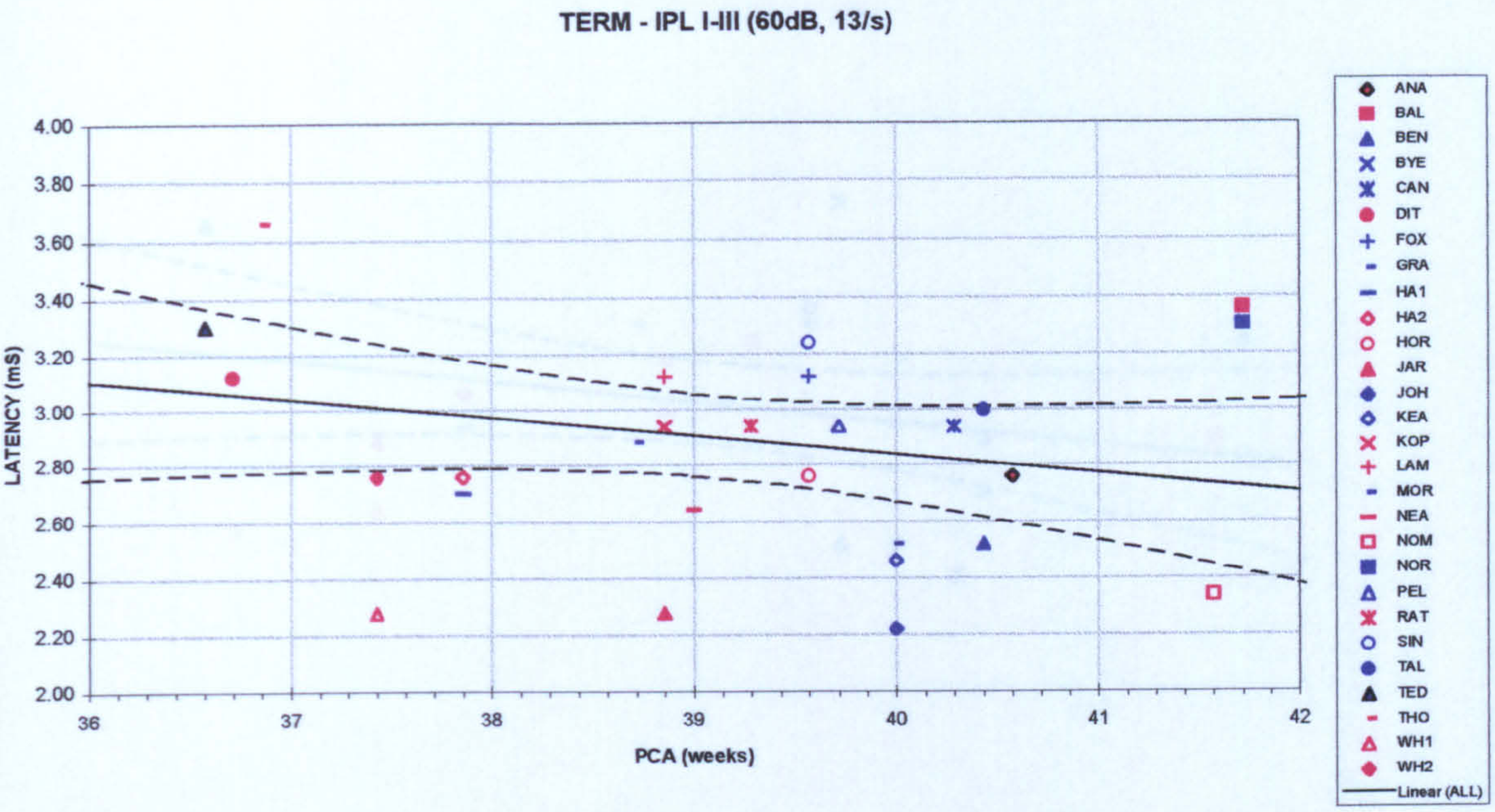


r^2=0.25 n=28 P<0.01

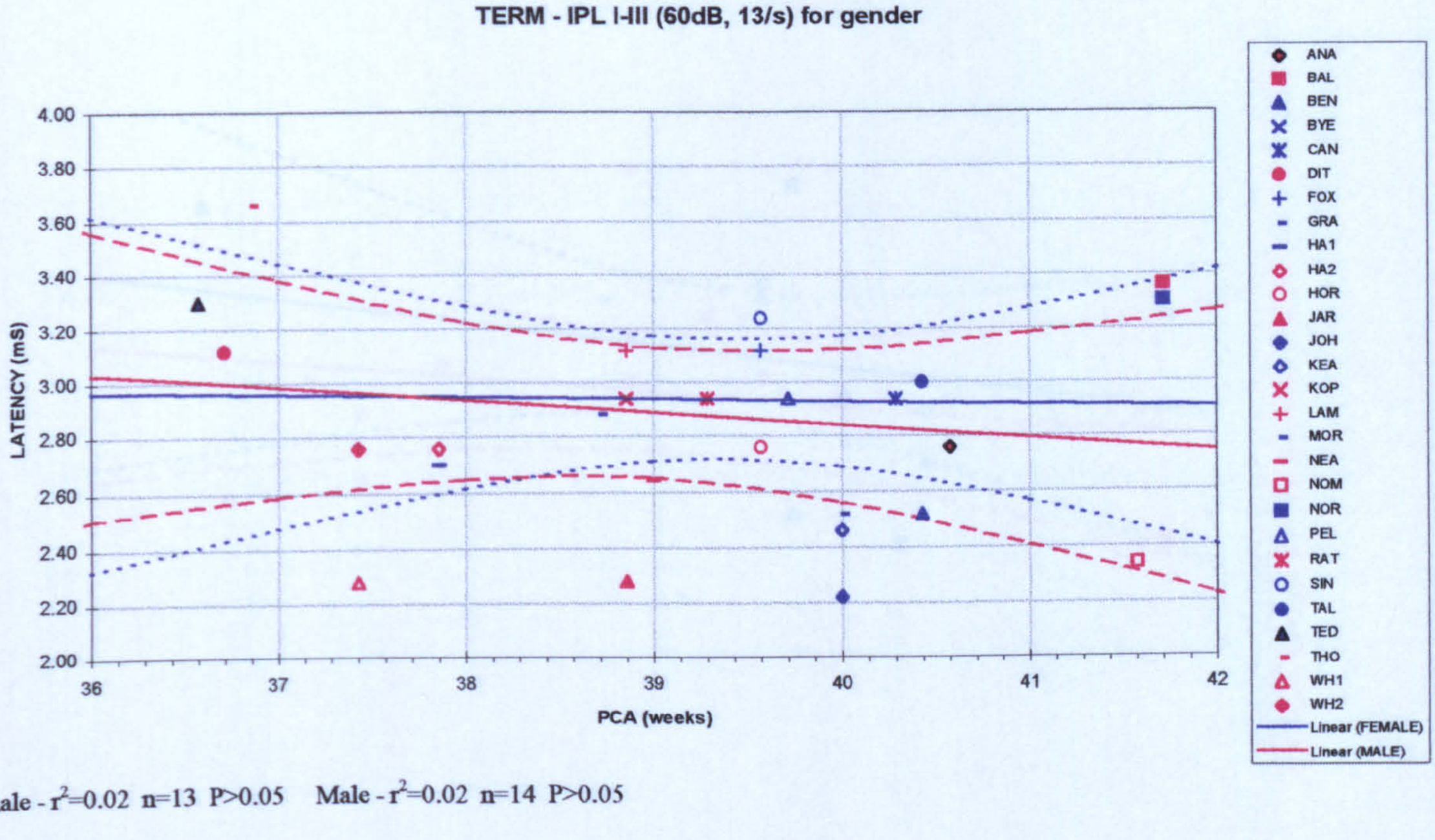


Female - r^2=0.16 n=14 P>0.05 Male - r^2=0.24 n=14 P>0.05

Figure C10 a/b

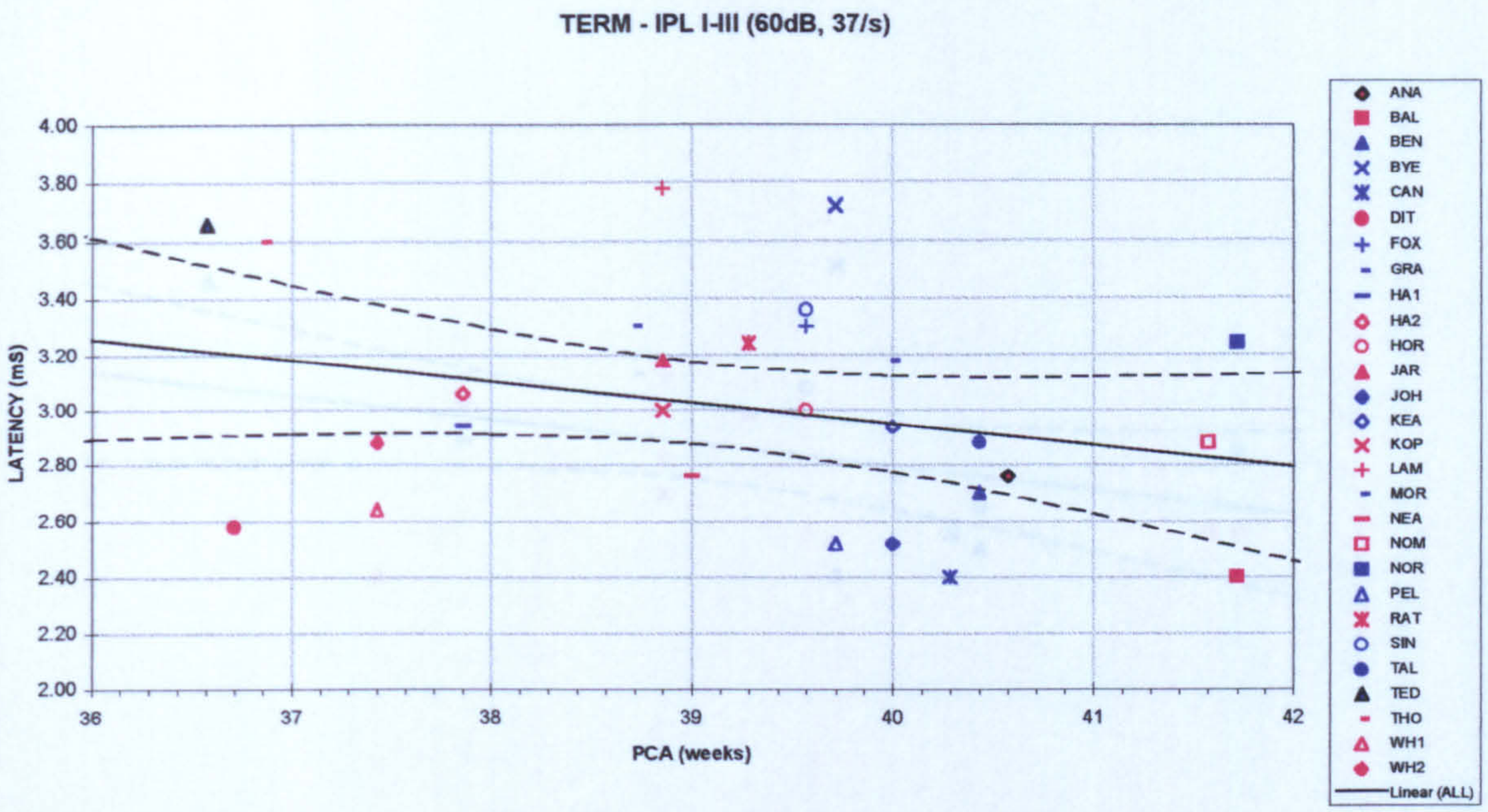


r^2=0.02 n=27 P>0.05

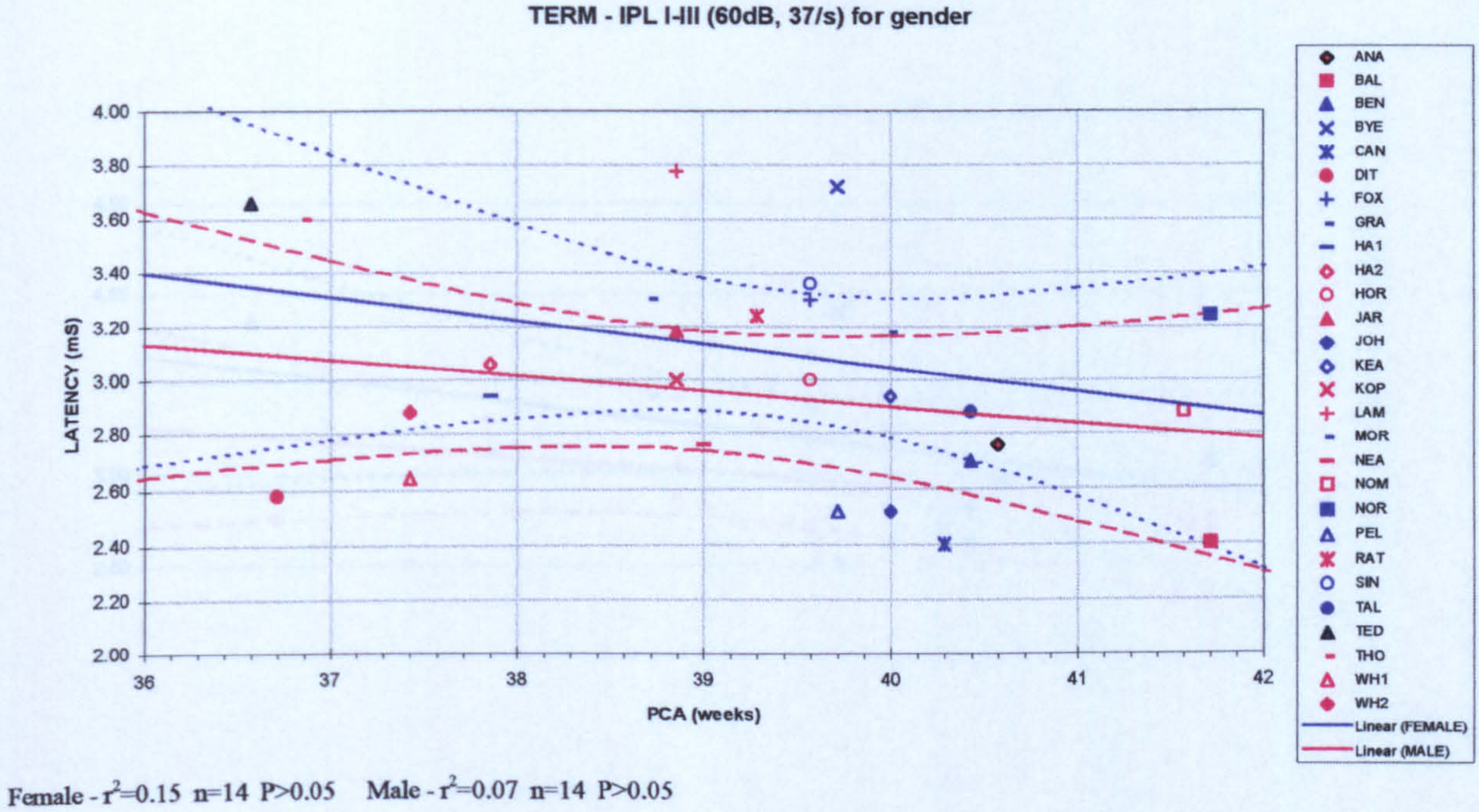


Female - r^2=0.02 n=13 P>0.05 Male - r^2=0.02 n=14 P>0.05

Figure C11 a/b

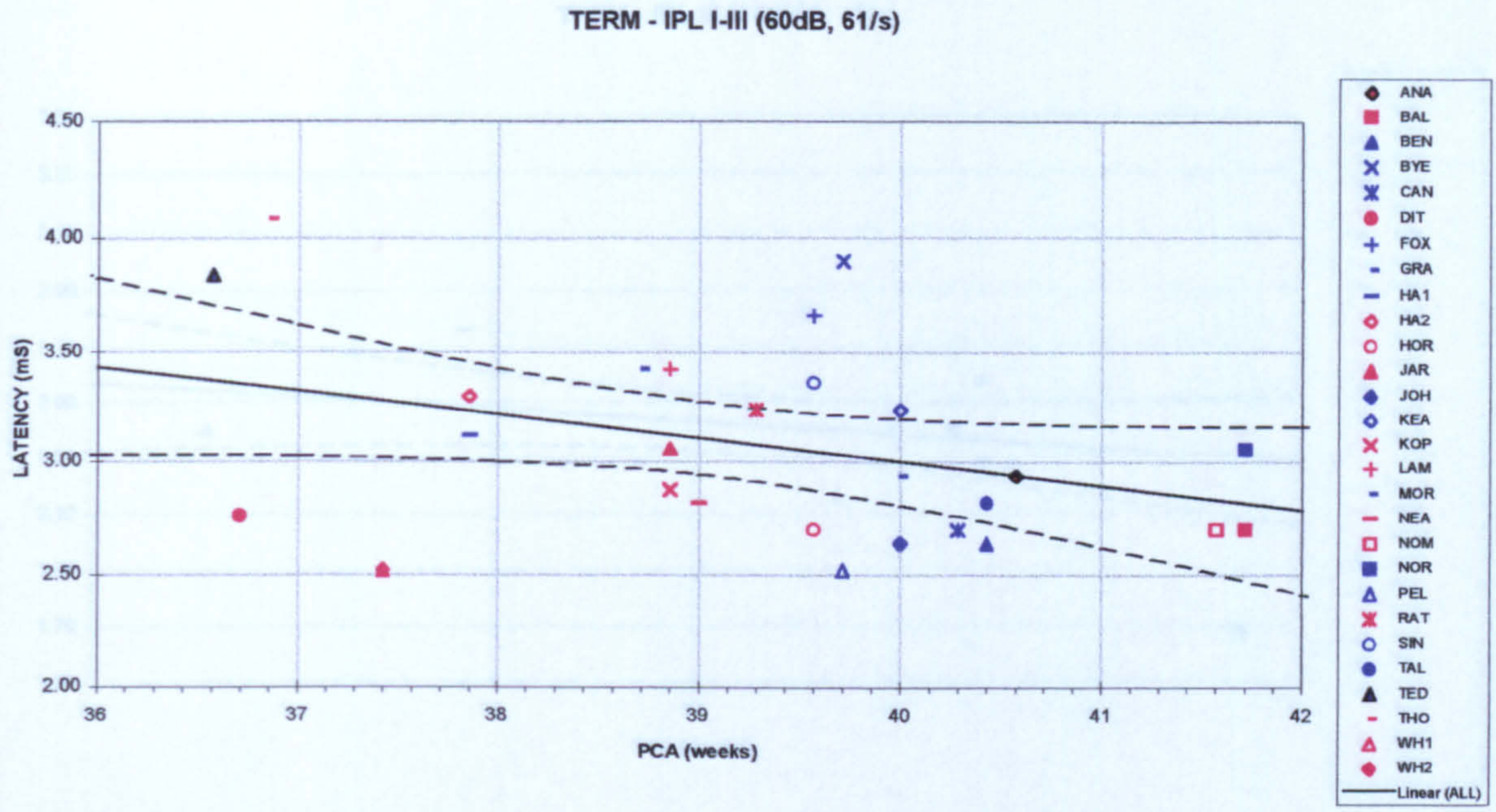


r^2=0.08 n=28 P>0.05

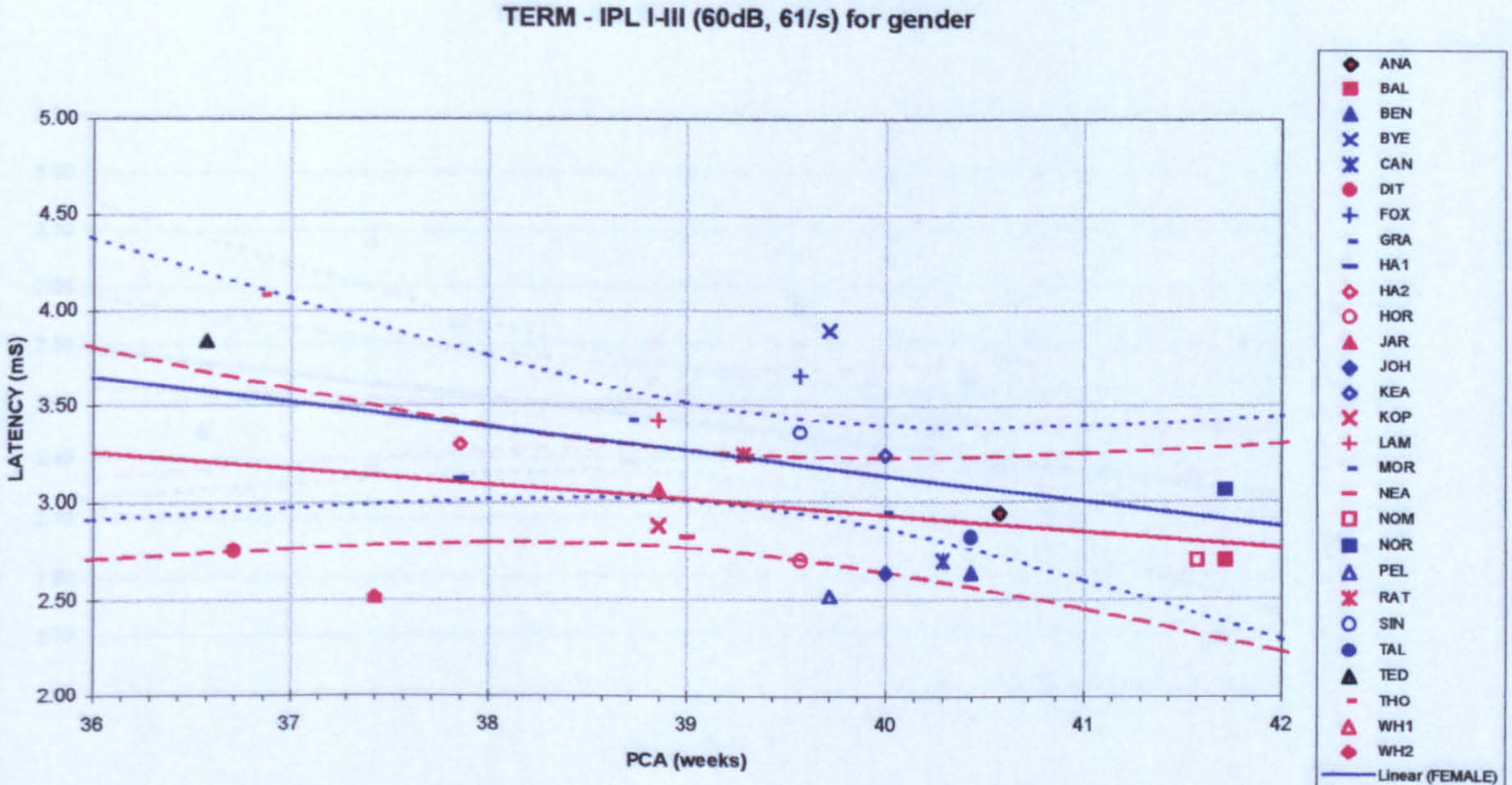


Female - r^2=0.15 n=14 P>0.05 Male - r^2=0.07 n=14 P>0.05

Figure C12 a/b

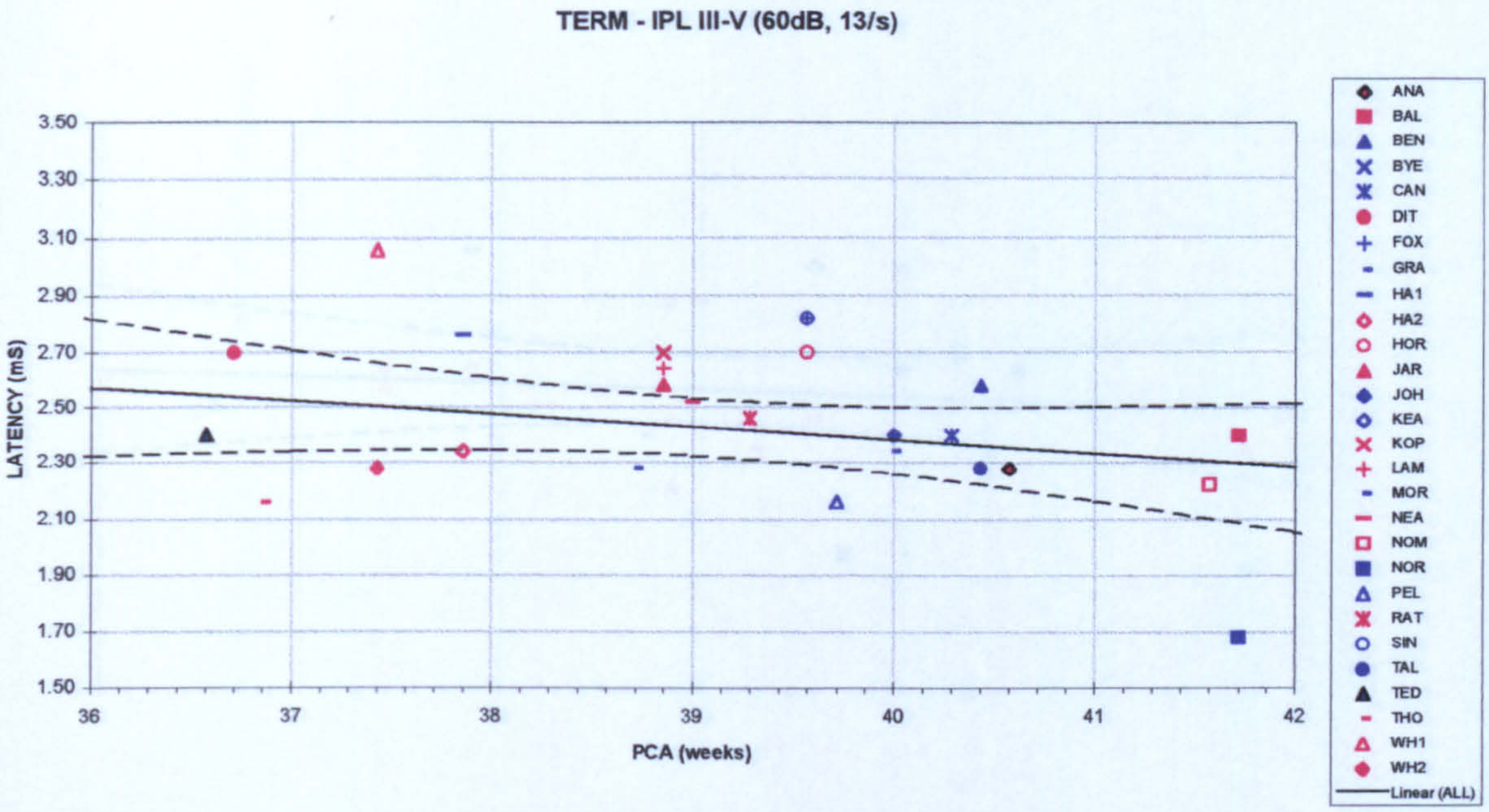


r^2=0.10 n=28 P>0.05

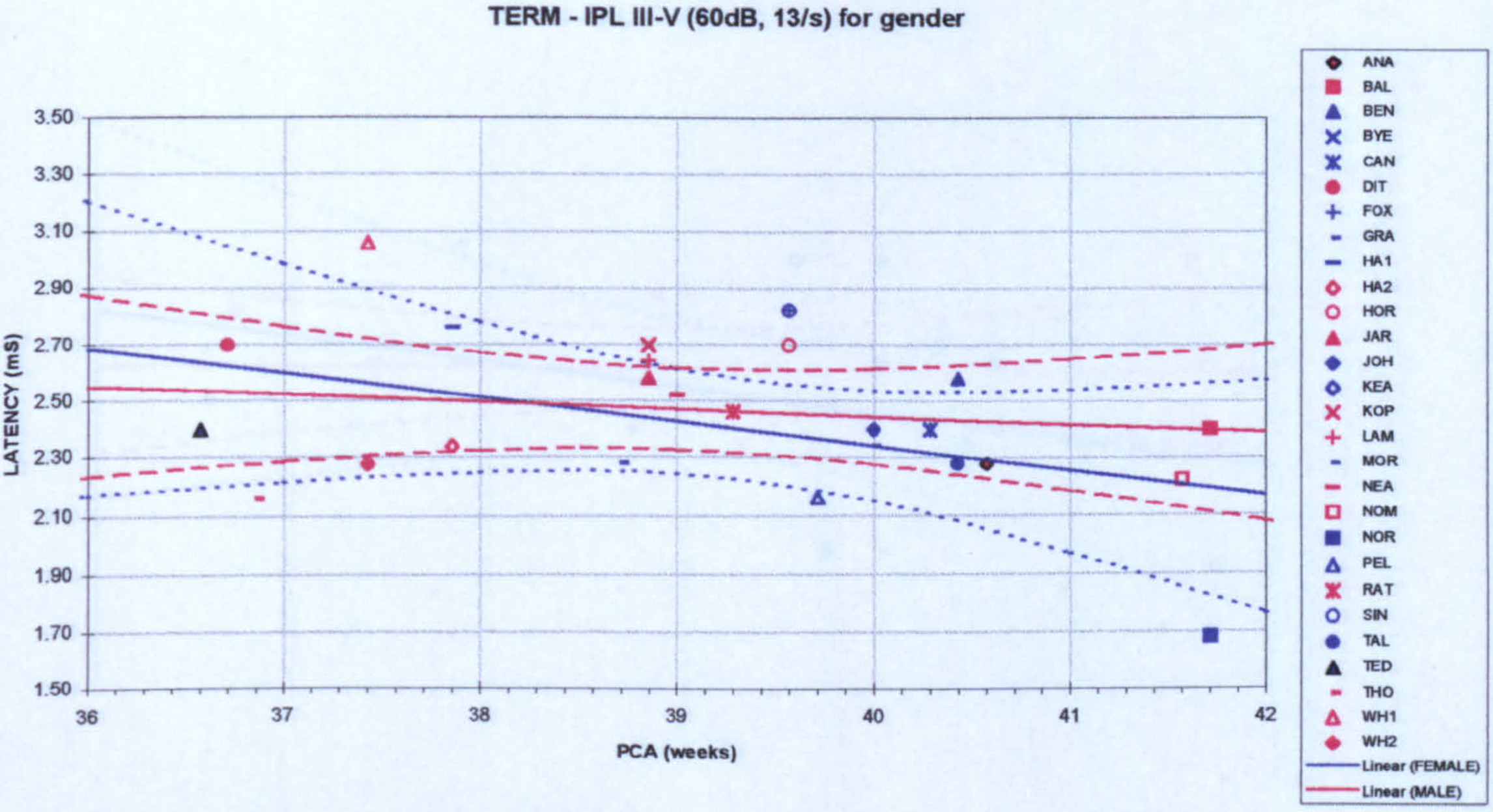


Female - r^2=0.26 n=14 P>0.05 Male - r^2=0.08 n=14 P>0.05

Figure C13 a/b

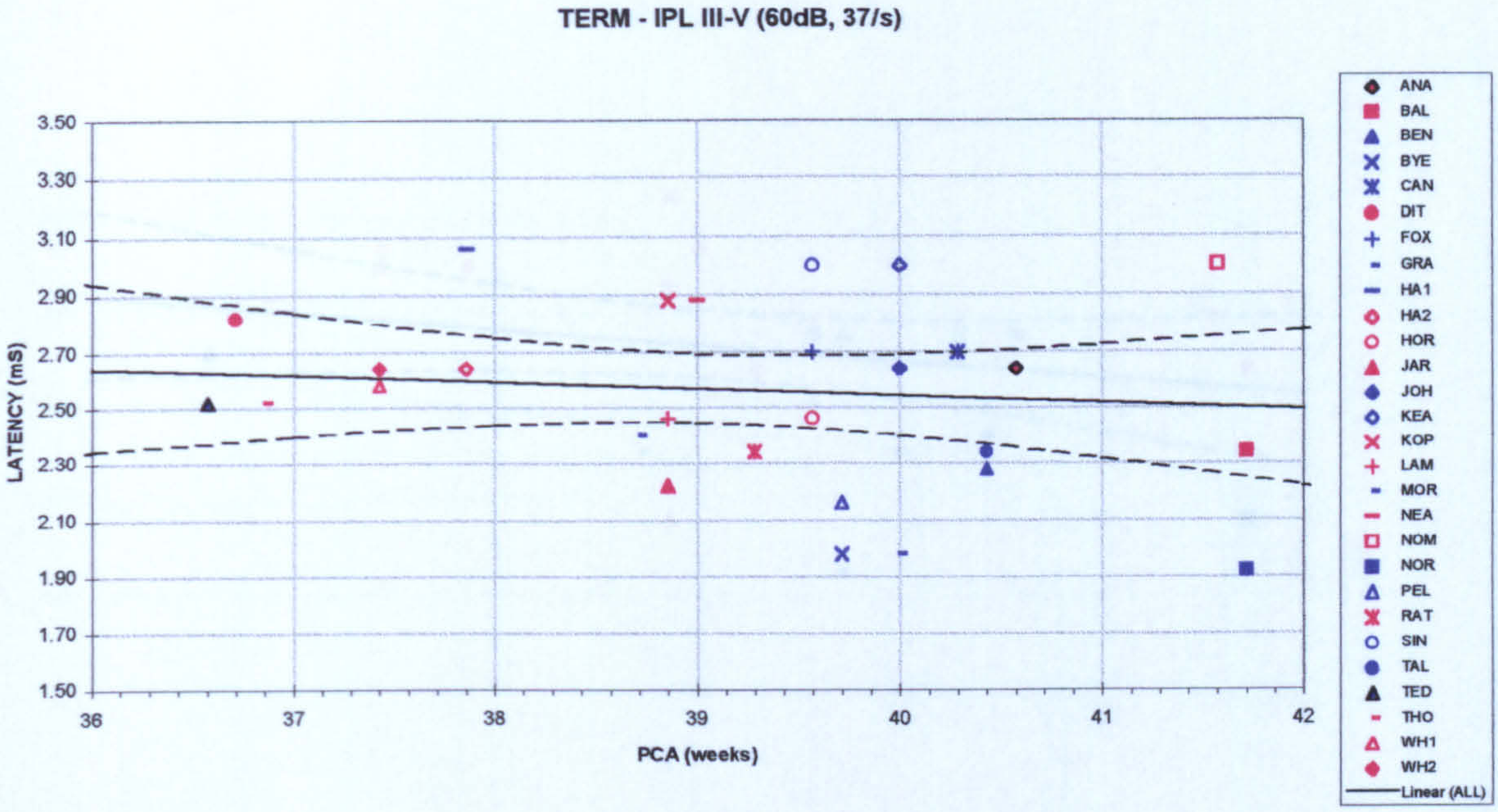


r^2=0.14 n=27 P>0.05

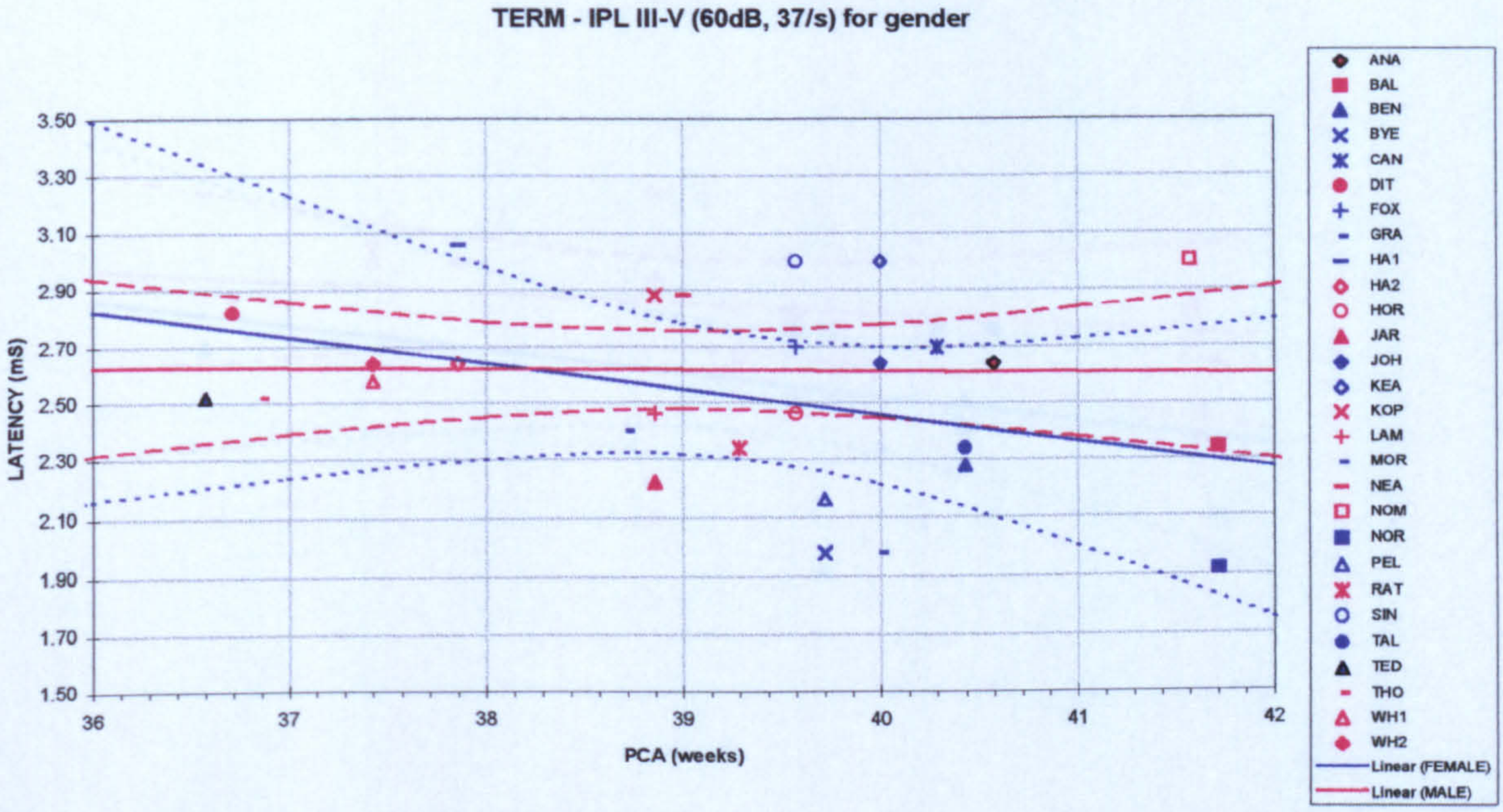


Female - r^2=0.19 n=13 P>0.05 Male - r^2=0.07 n=14 P>0.05

Figure C14 a/b

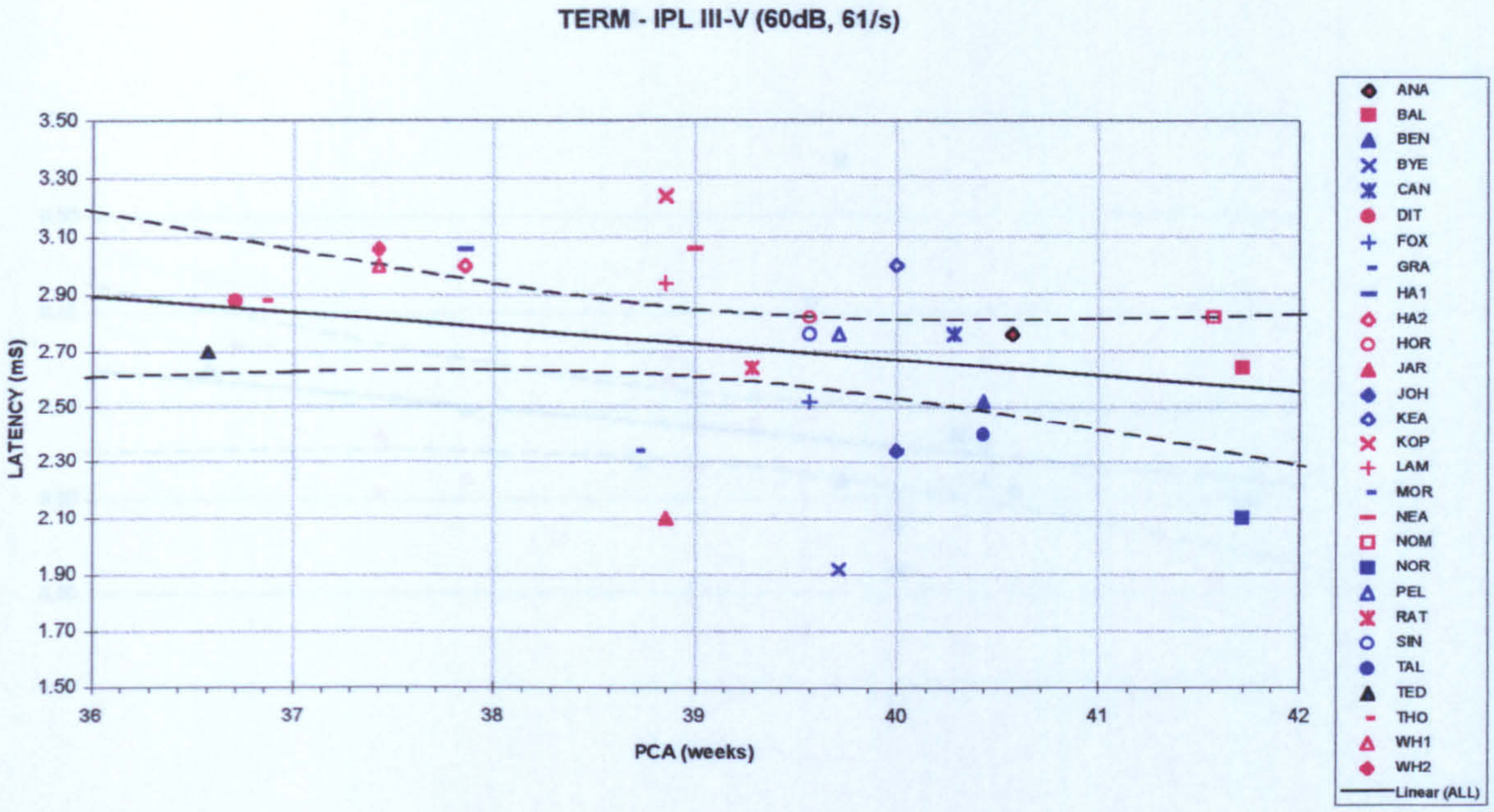


r^2=0.06 n=28 P>0.05

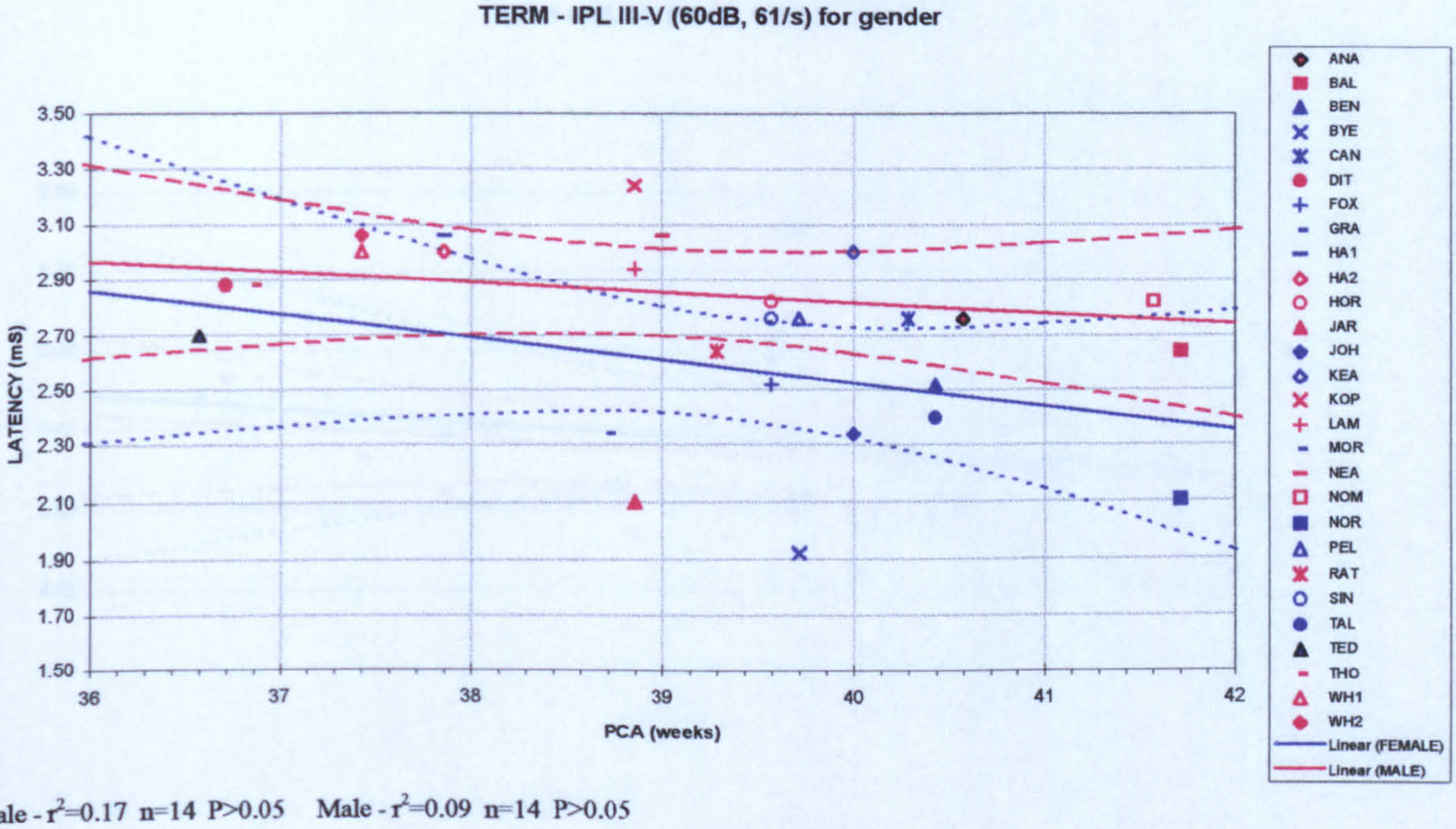


Female - r^2=0.15 n=14 P>0.05 Male - r^2=0.00 n=14 P>0.05

Figure C15 a/b

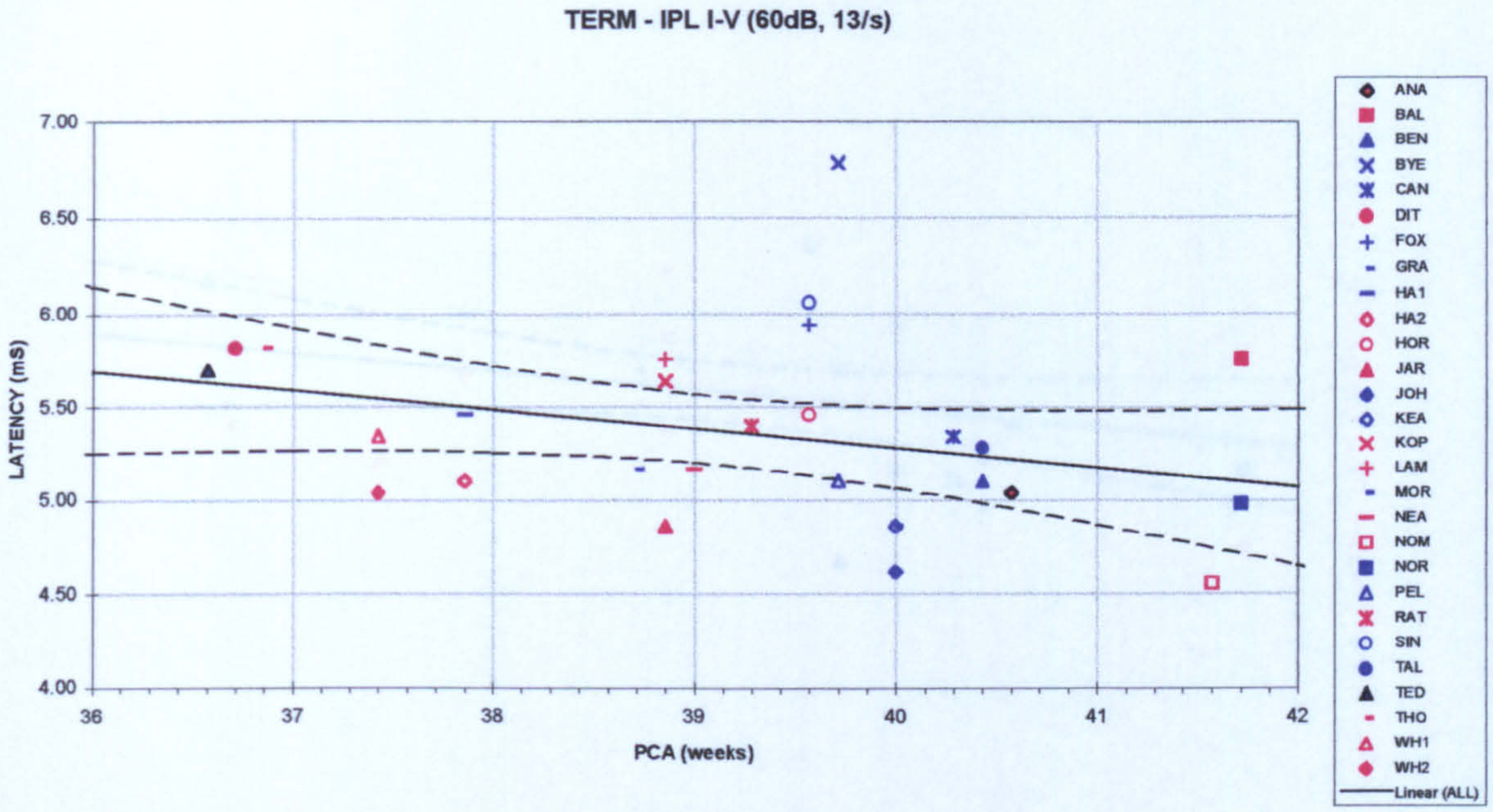


r^2=0.17 n=28 P<0.05

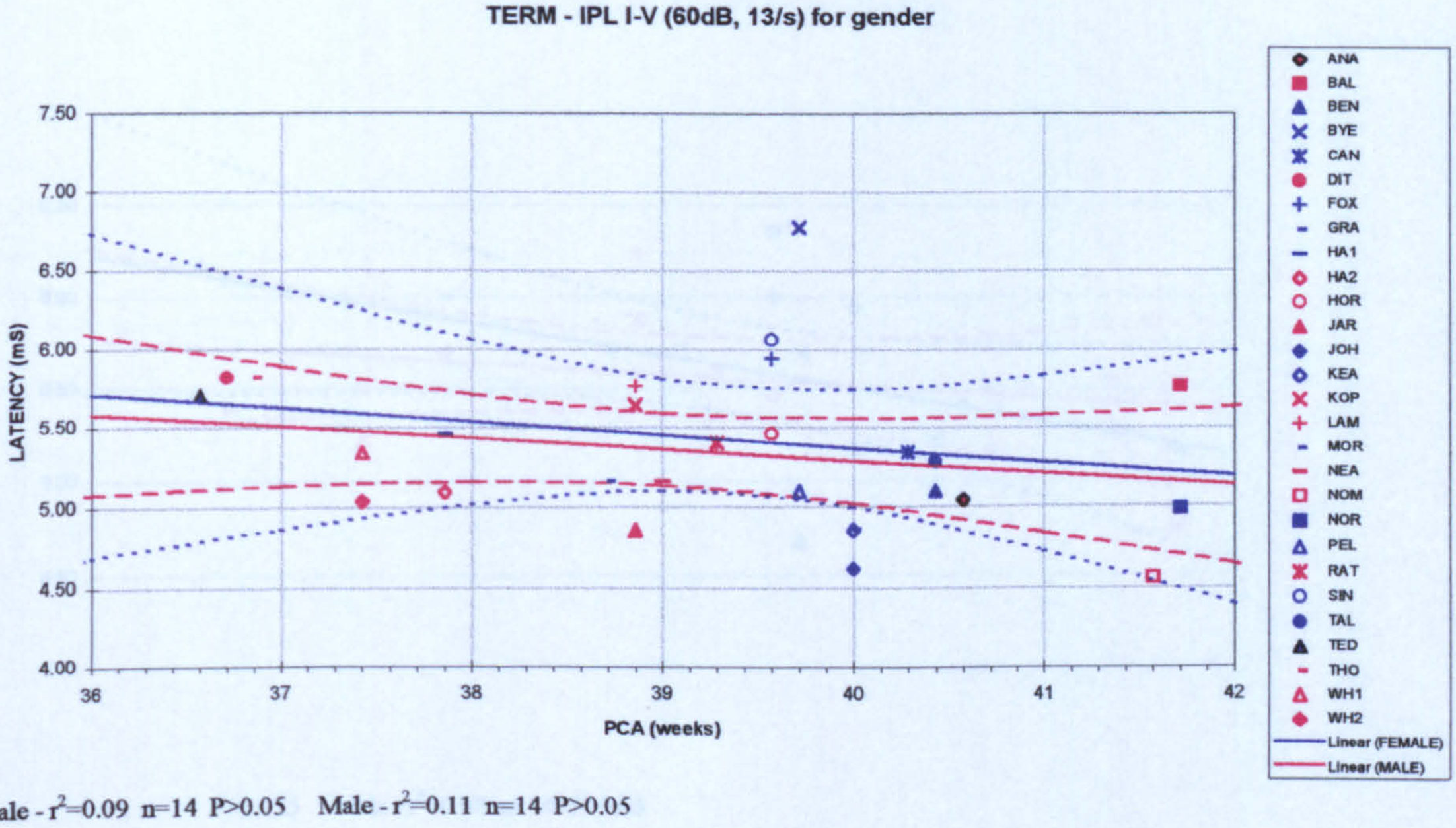


Female - r^2=0.17 n=14 P>0.05 Male - r^2=0.09 n=14 P>0.05

Figure C16 a/b

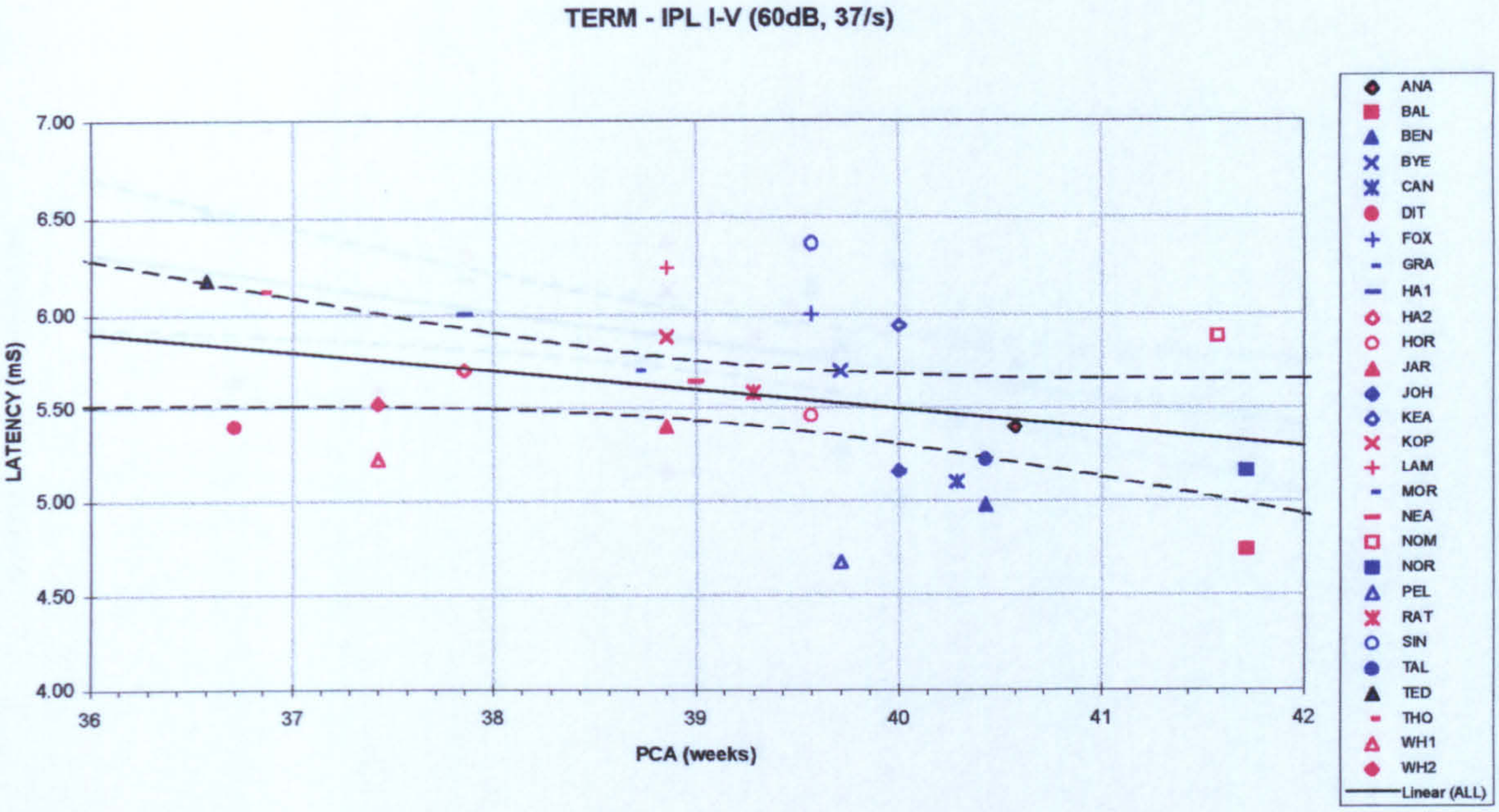


r^2=0.08 n=28 P>0.05

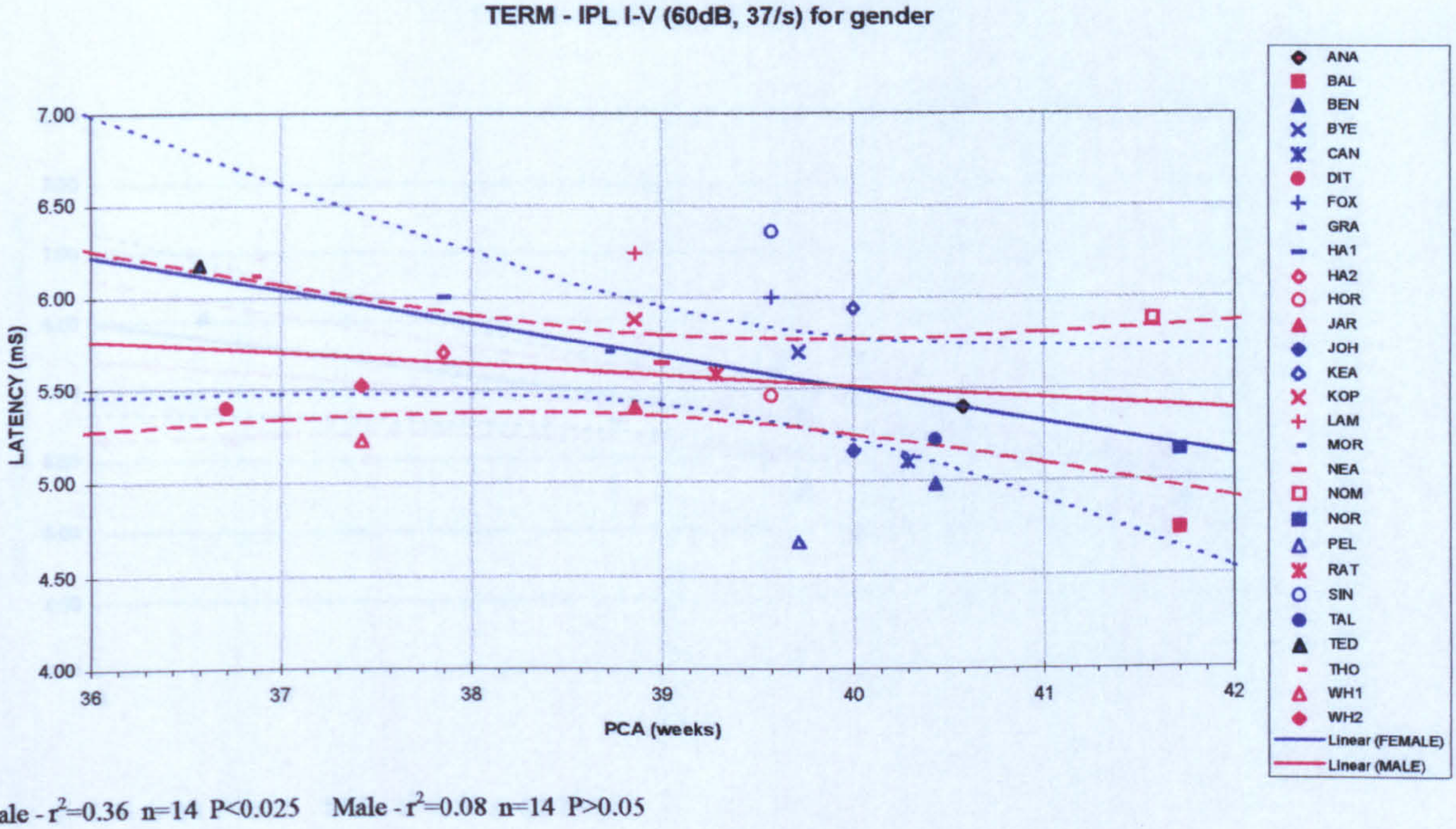


Female - r^2=0.09 n=14 P>0.05 Male - r^2=0.11 n=14 P>0.05

Figure C17 a/b

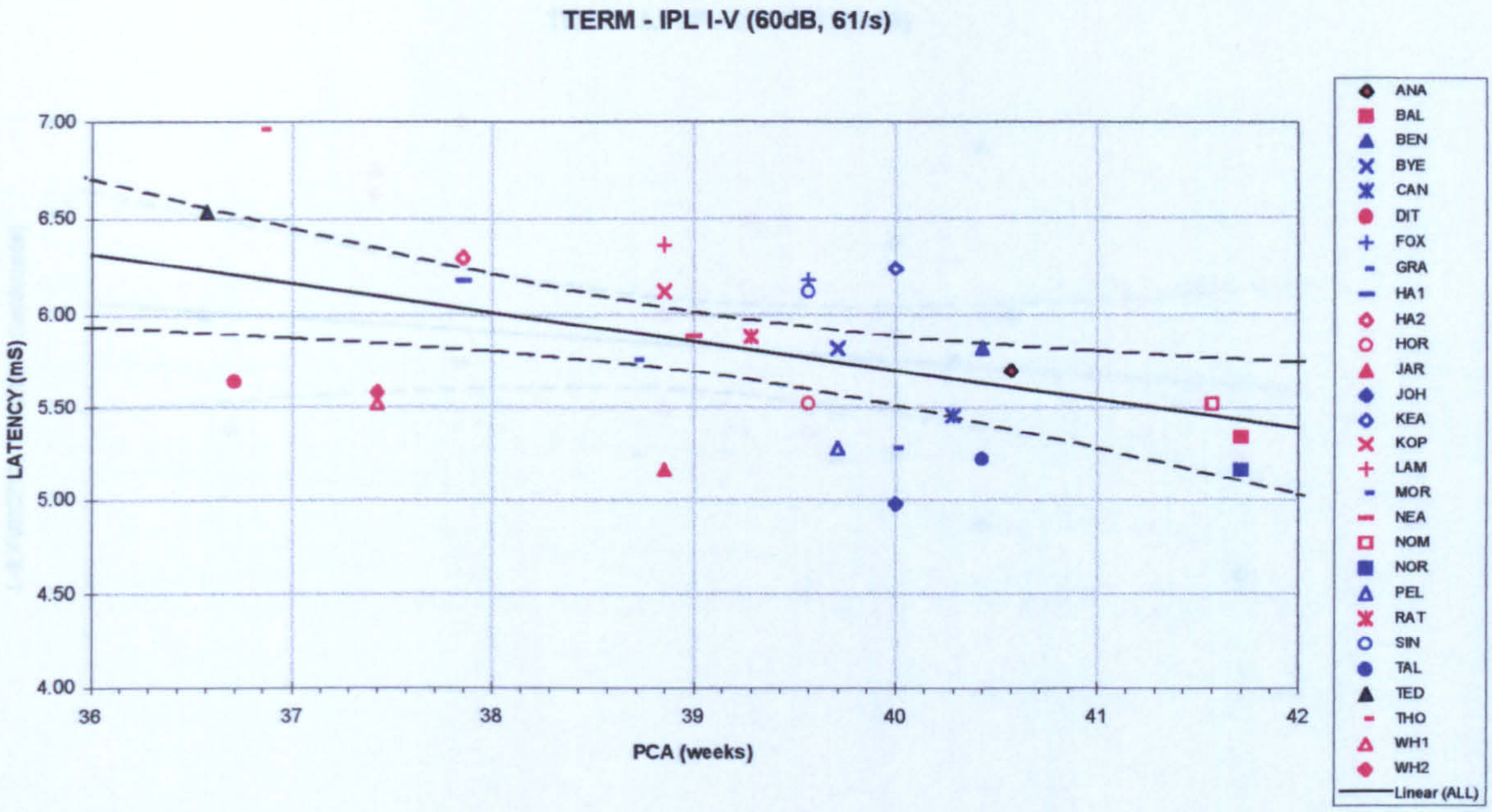


r^2=0.18 n=28 P<0.025

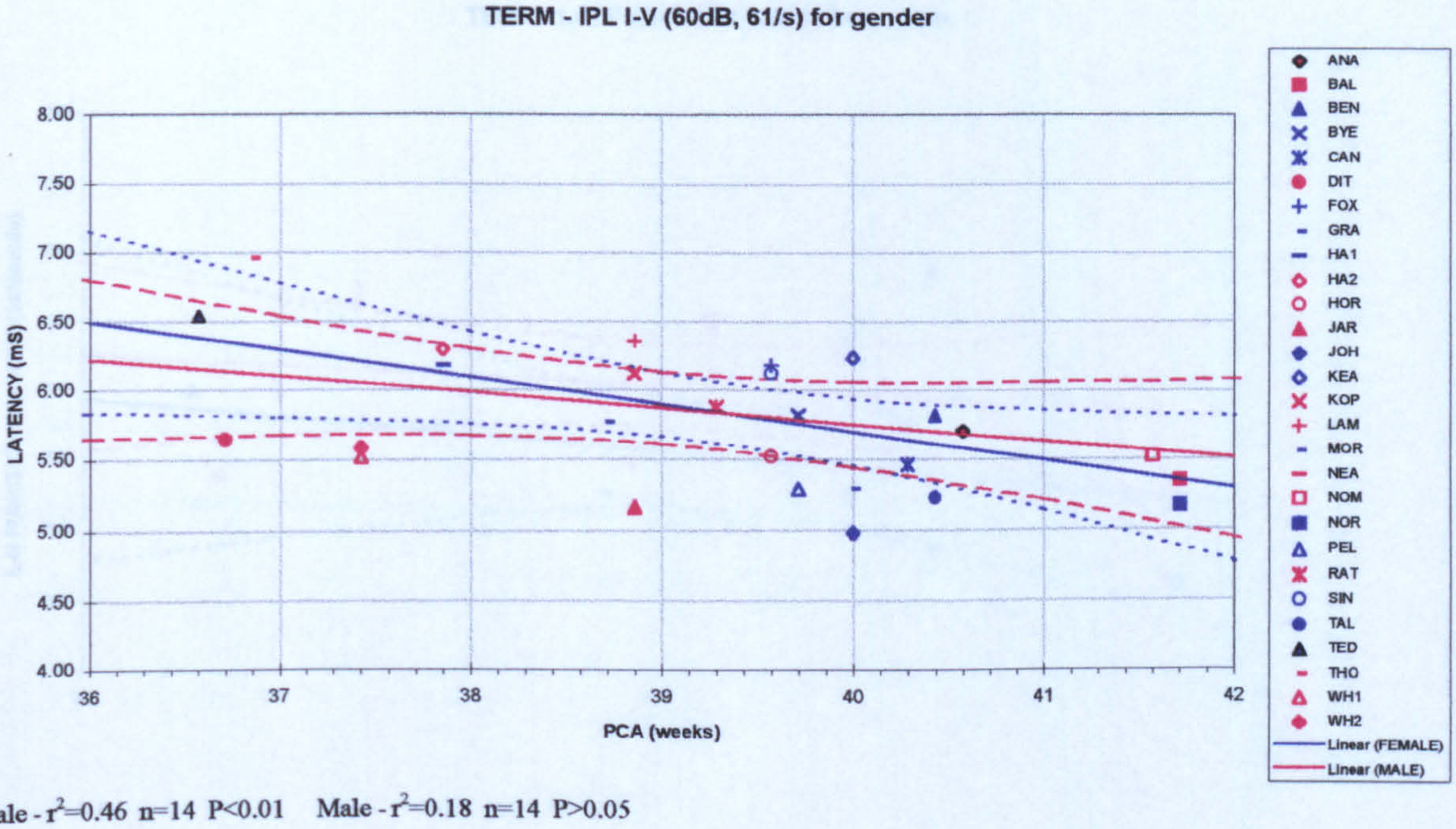


Female - r^2=0.36 n=14 P<0.025 Male - r^2=0.08 n=14 P>0.05

Figure C18 a/b

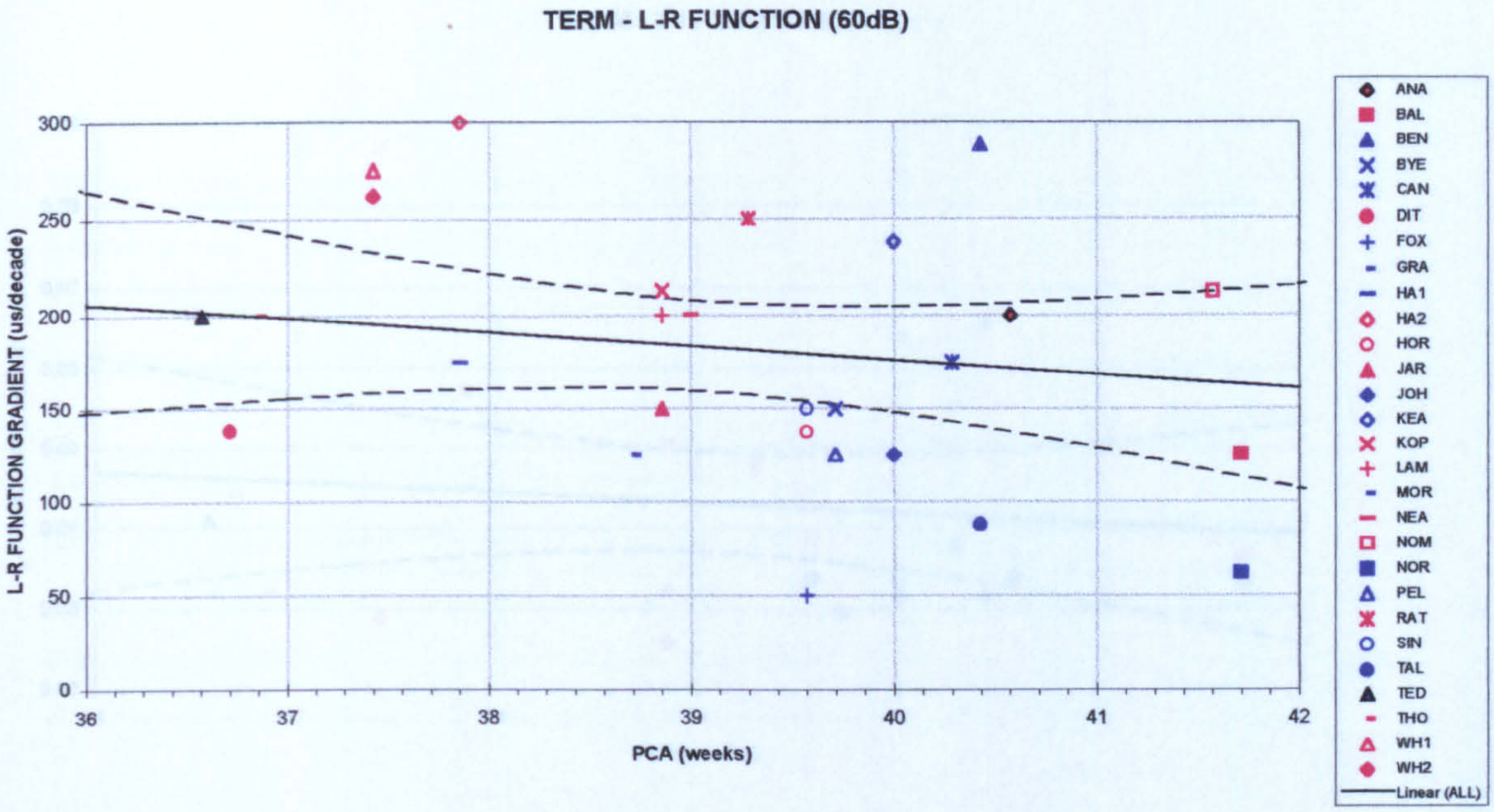


r^2=0.29 n=28 P<0.005

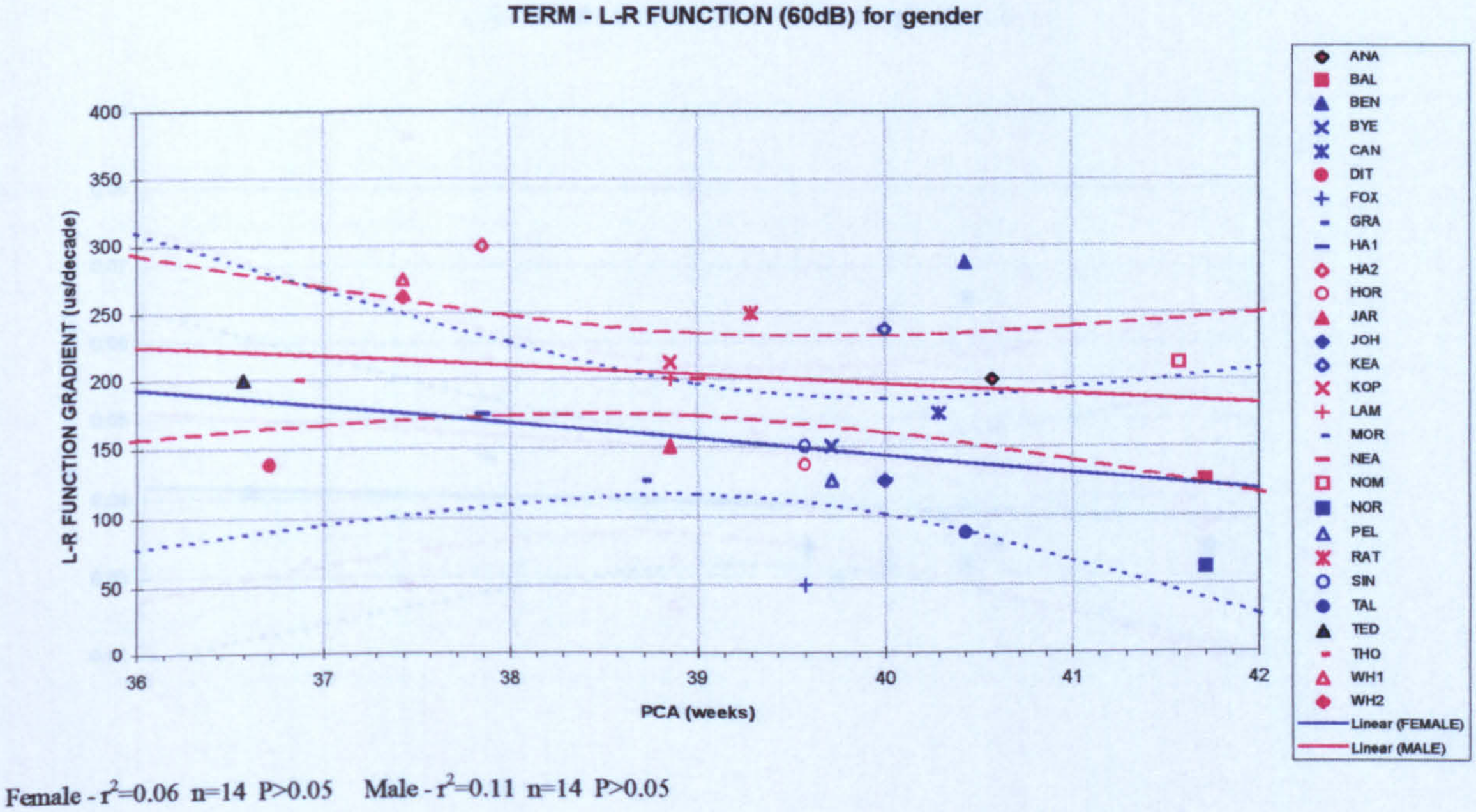


Female - r^2=0.46 n=14 P<0.01 Male - r^2=0.18 n=14 P>0.05

Figure C19 a/b

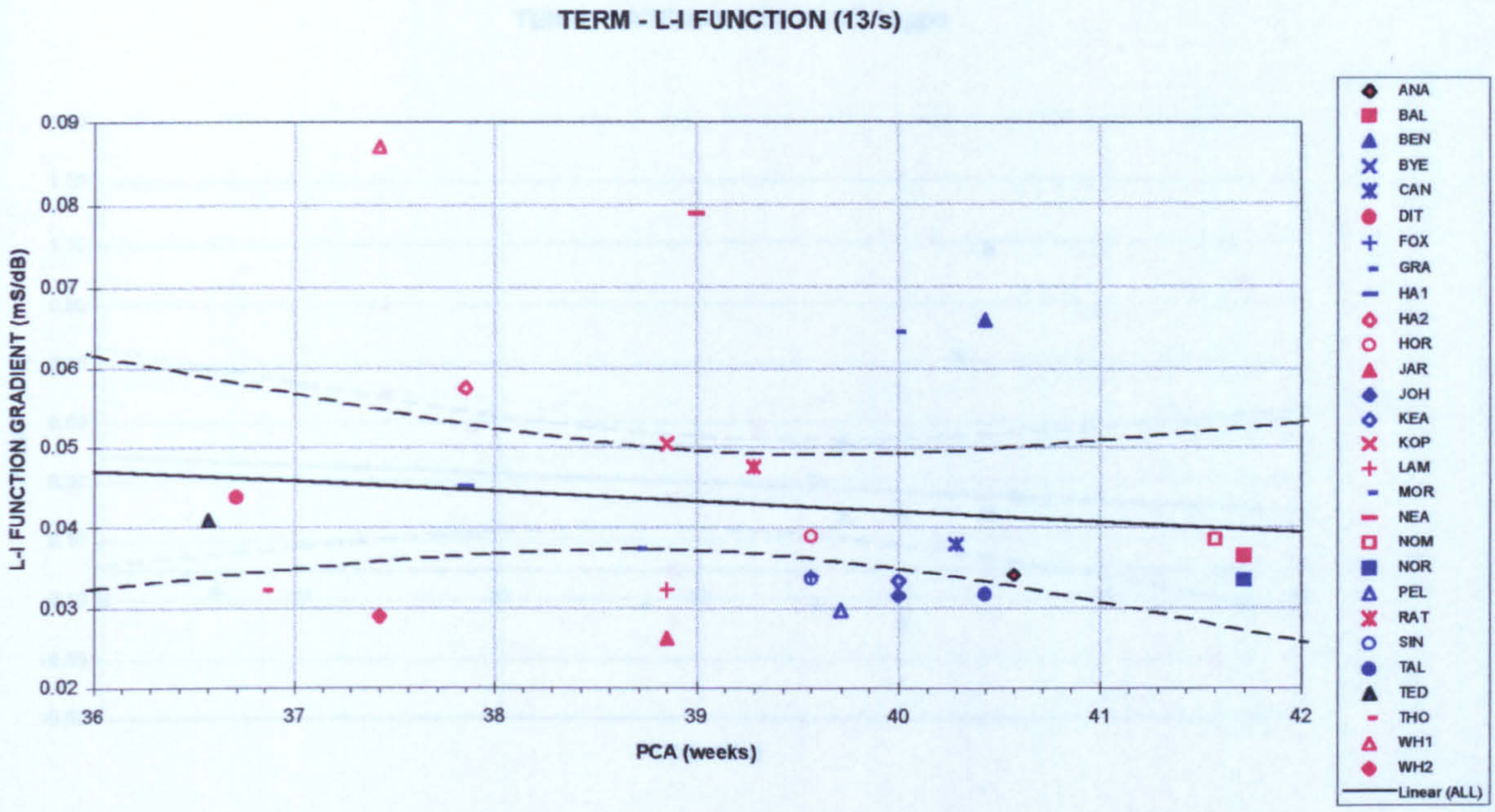


r^2=0.13 n=28 P>0.05

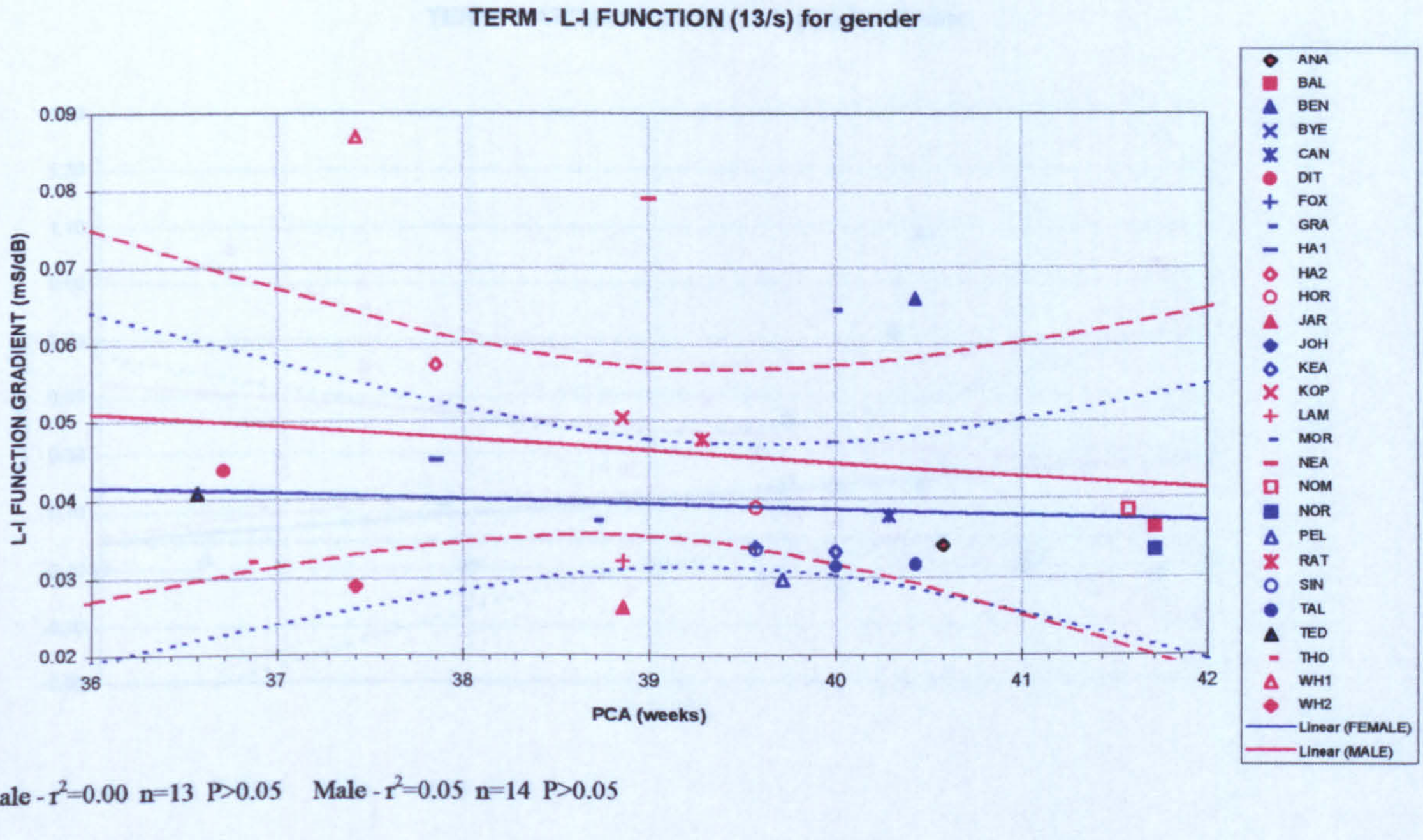


Female - r^2=0.06 n=14 P>0.05 Male - r^2=0.11 n=14 P>0.05

Figure C20 a/b

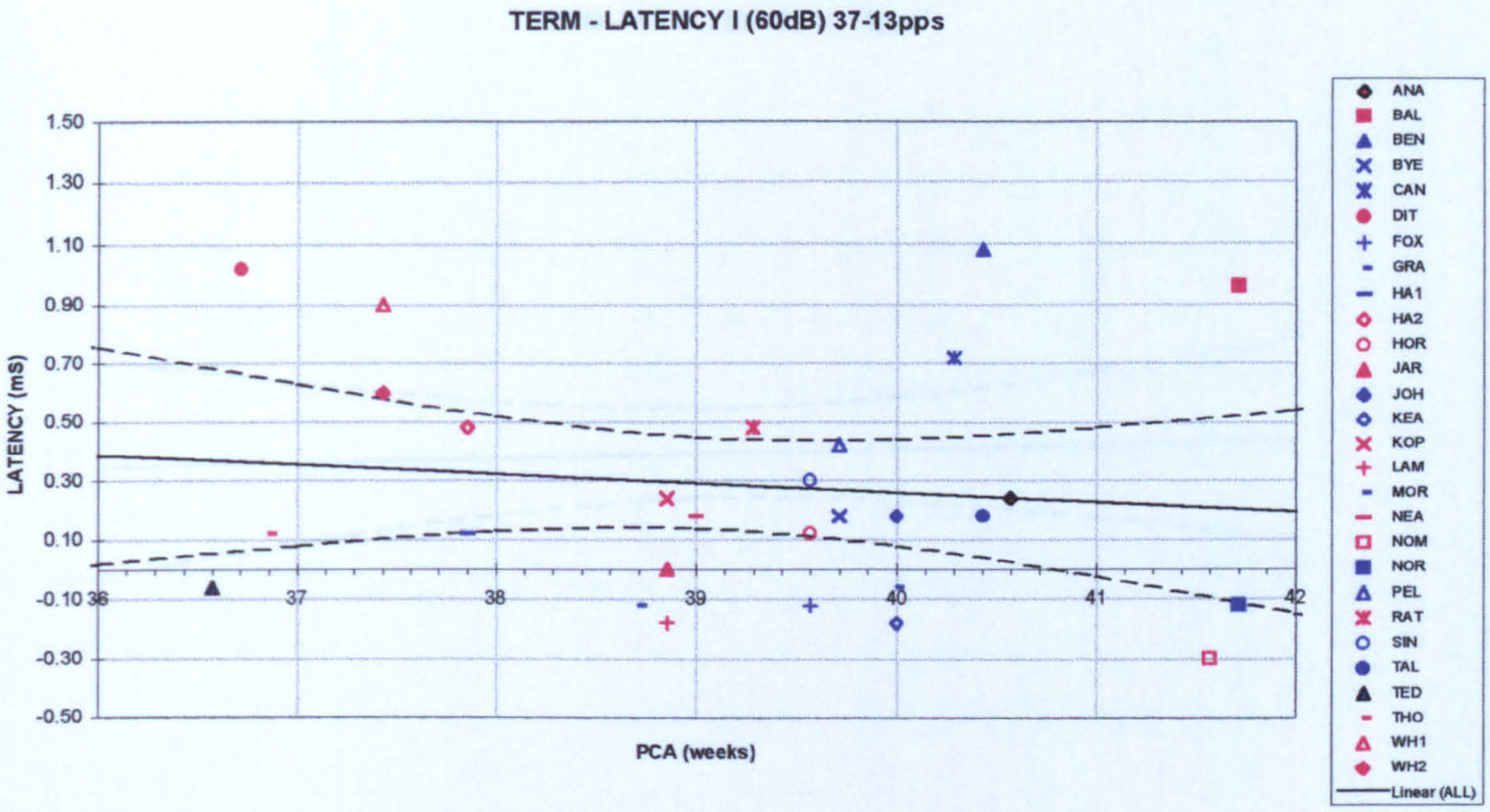


r^2=0.04 n=27 P>0.05

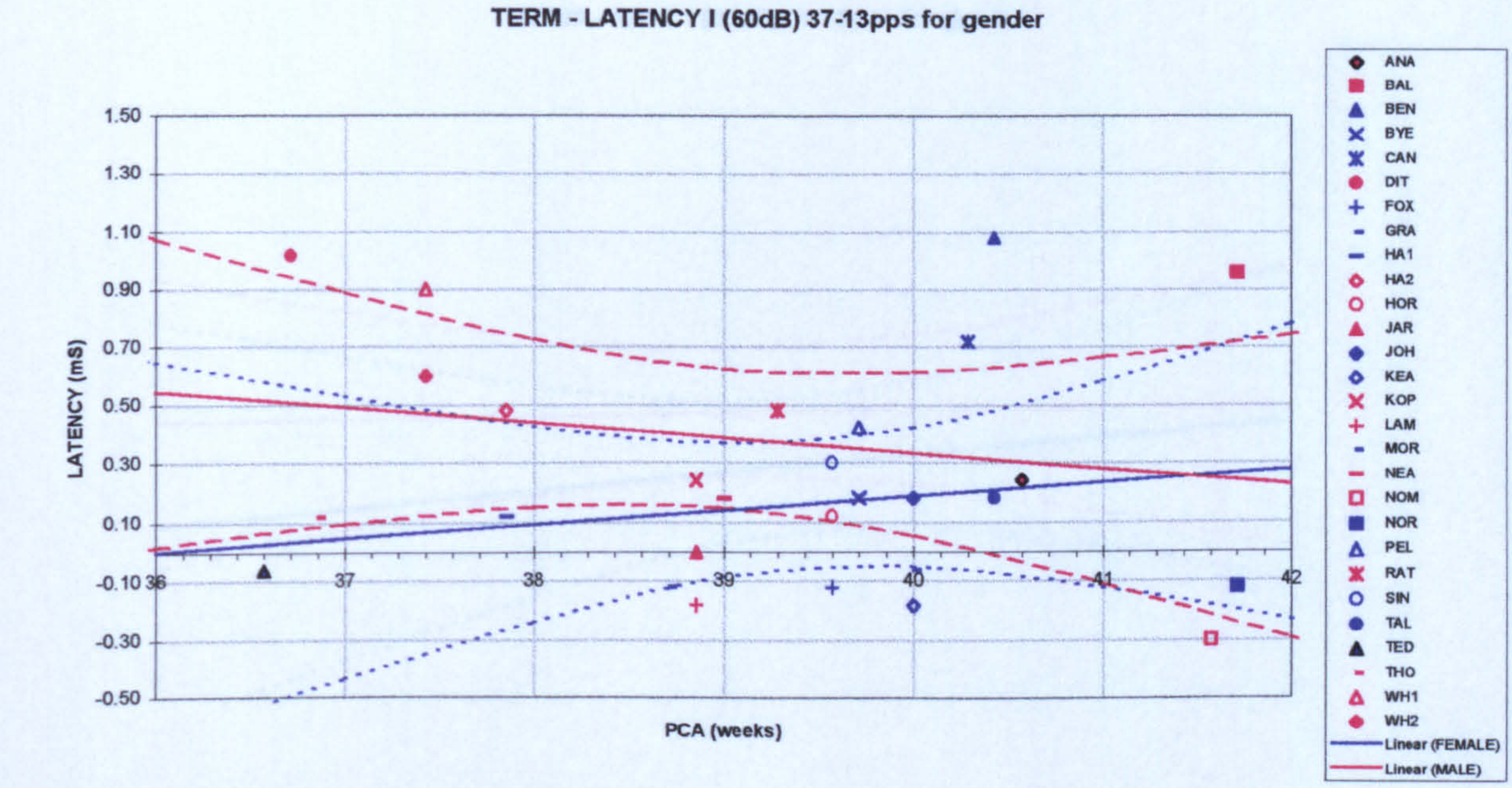


Female - r^2=0.00 n=13 P>0.05 Male - r^2=0.05 n=14 P>0.05

Figure C21 a/b

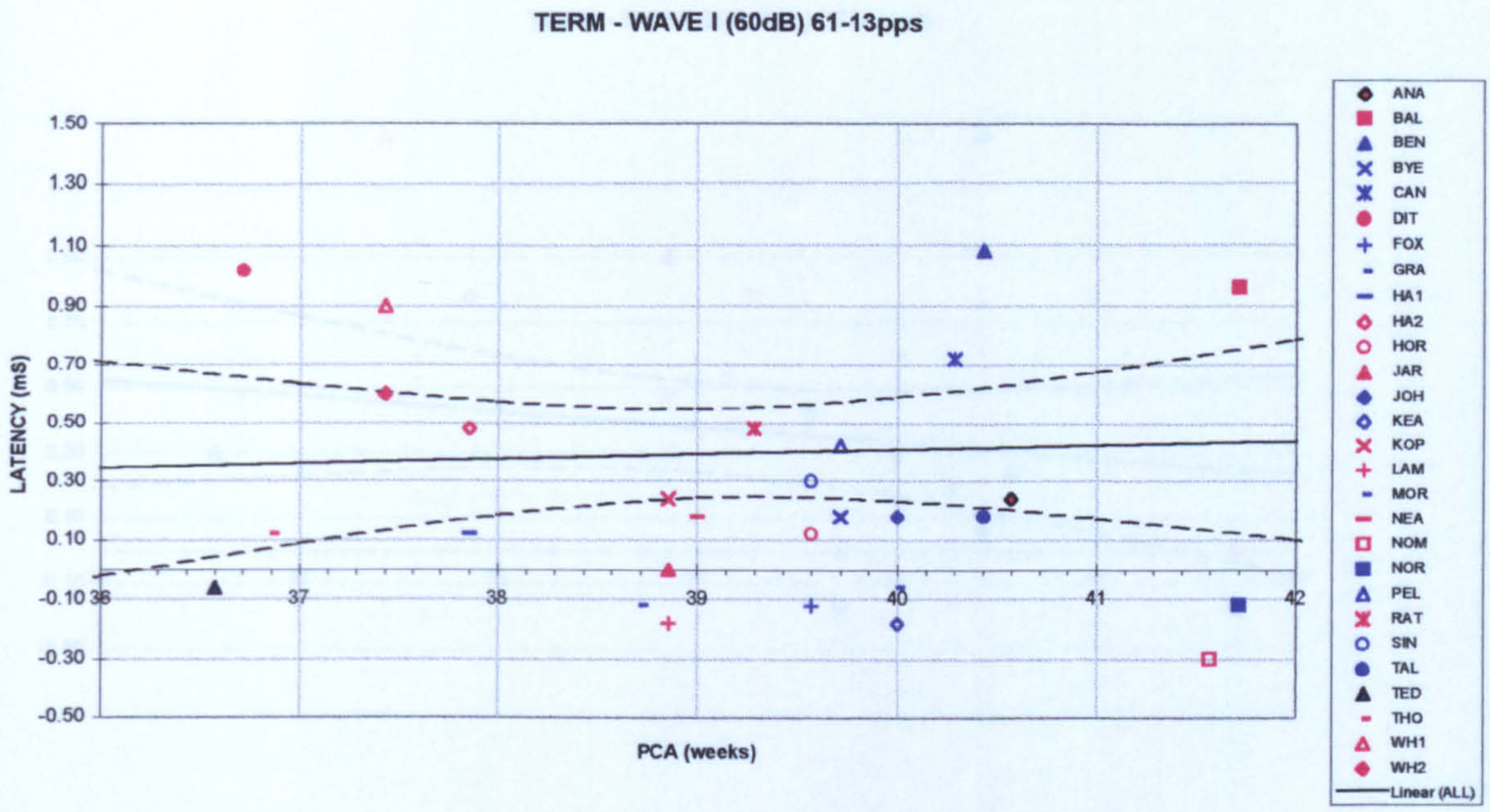


r^2=0.02 n=28 P>0.05

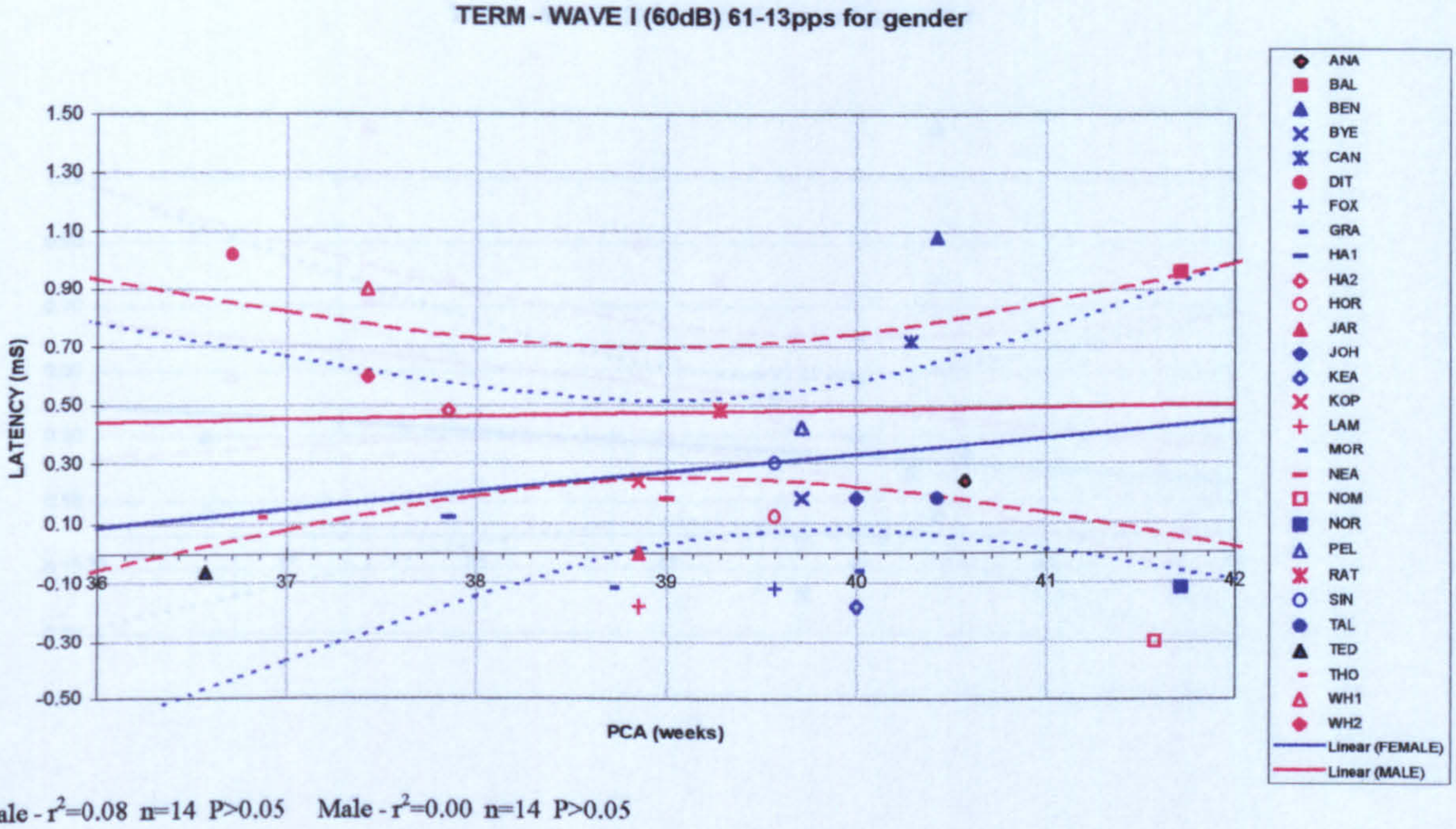


Female - r^2=0.06 n=14 P>0.05 Male - r^2=0.09 n=14 P>0.05

Figure C22 a/b

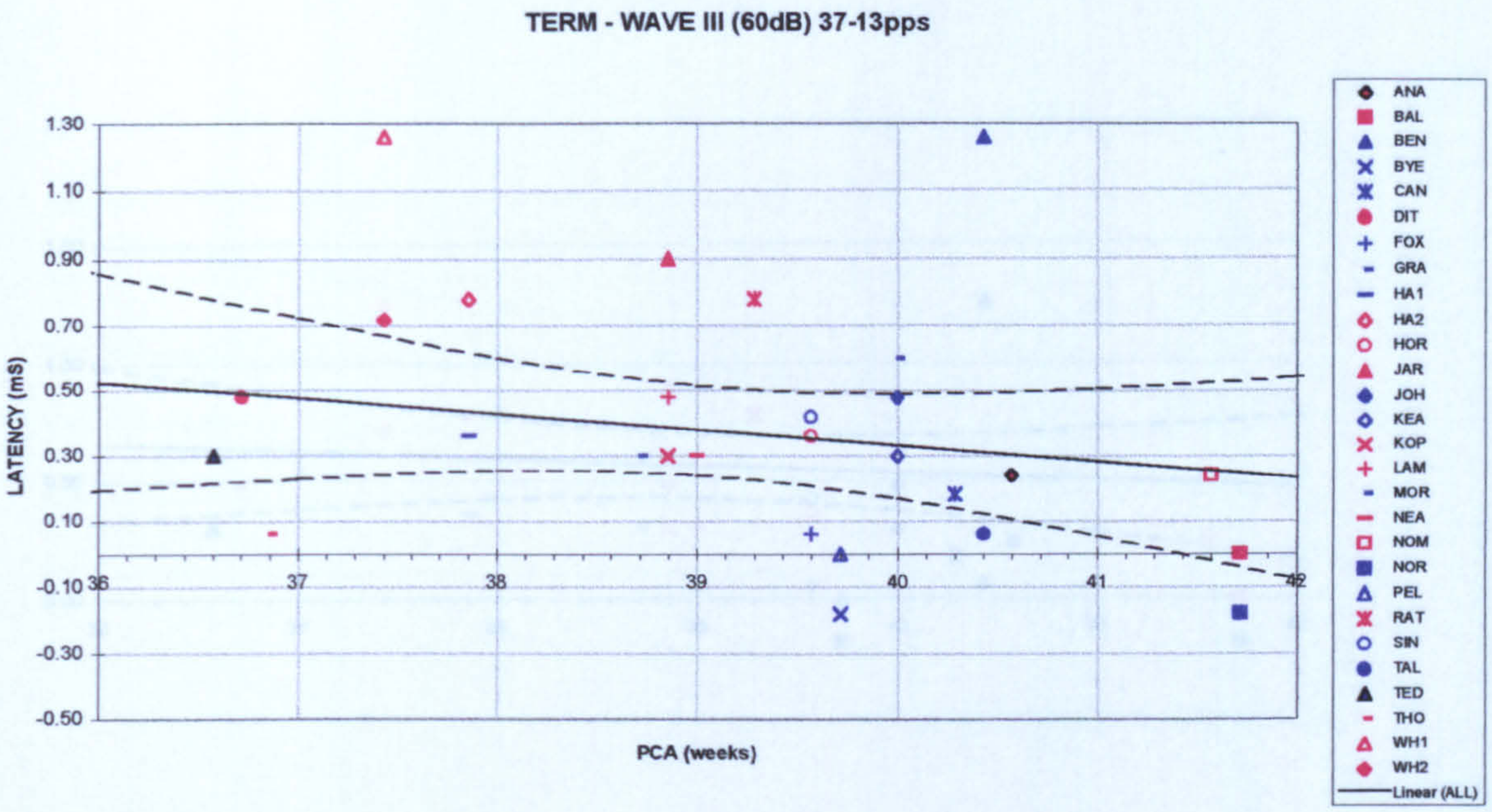


r^2=0.00 n=28 P>0.05

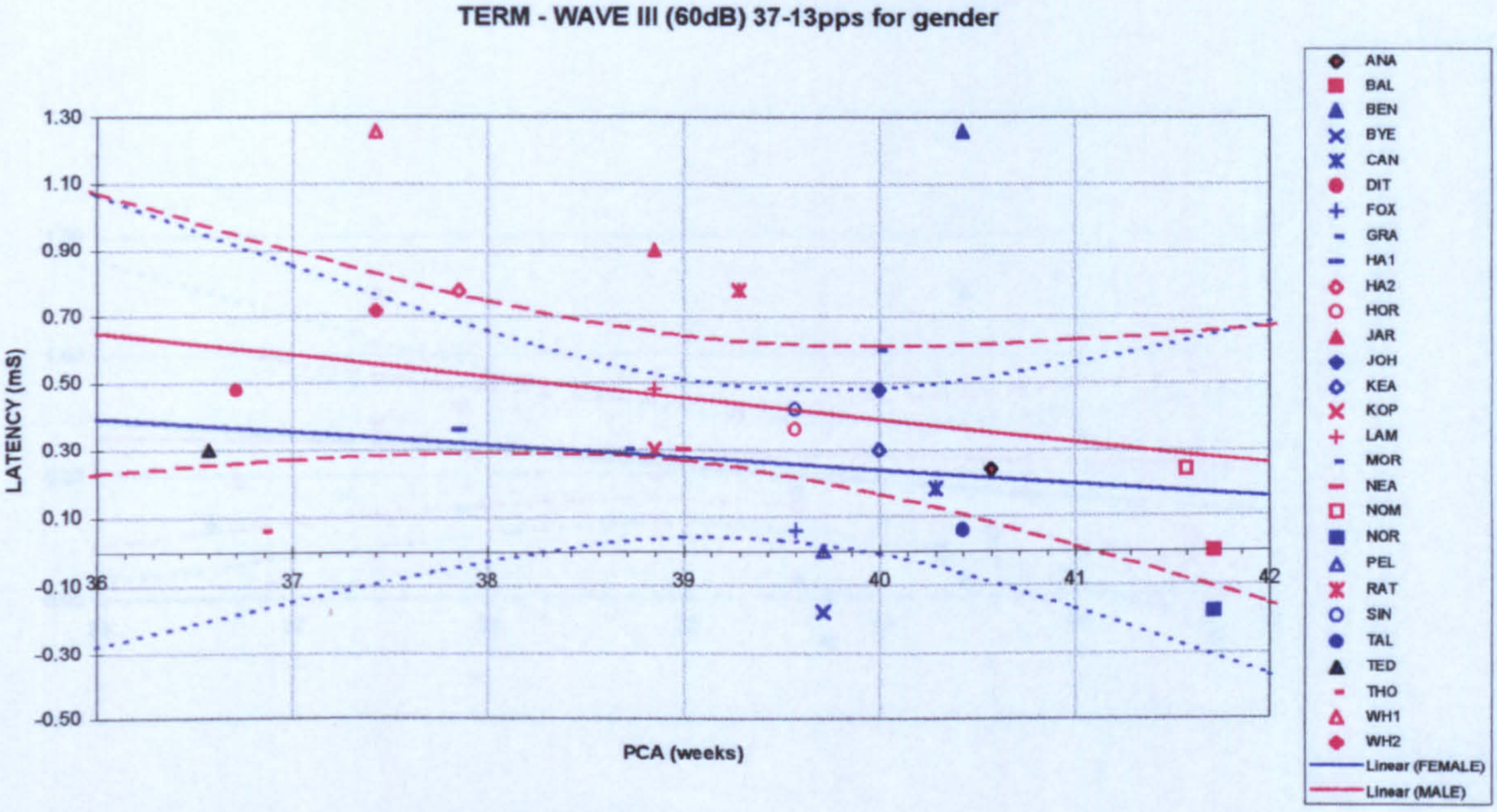


Female - r^2=0.08 n=14 P>0.05 Male - r^2=0.00 n=14 P>0.05

Figure C23 a/b

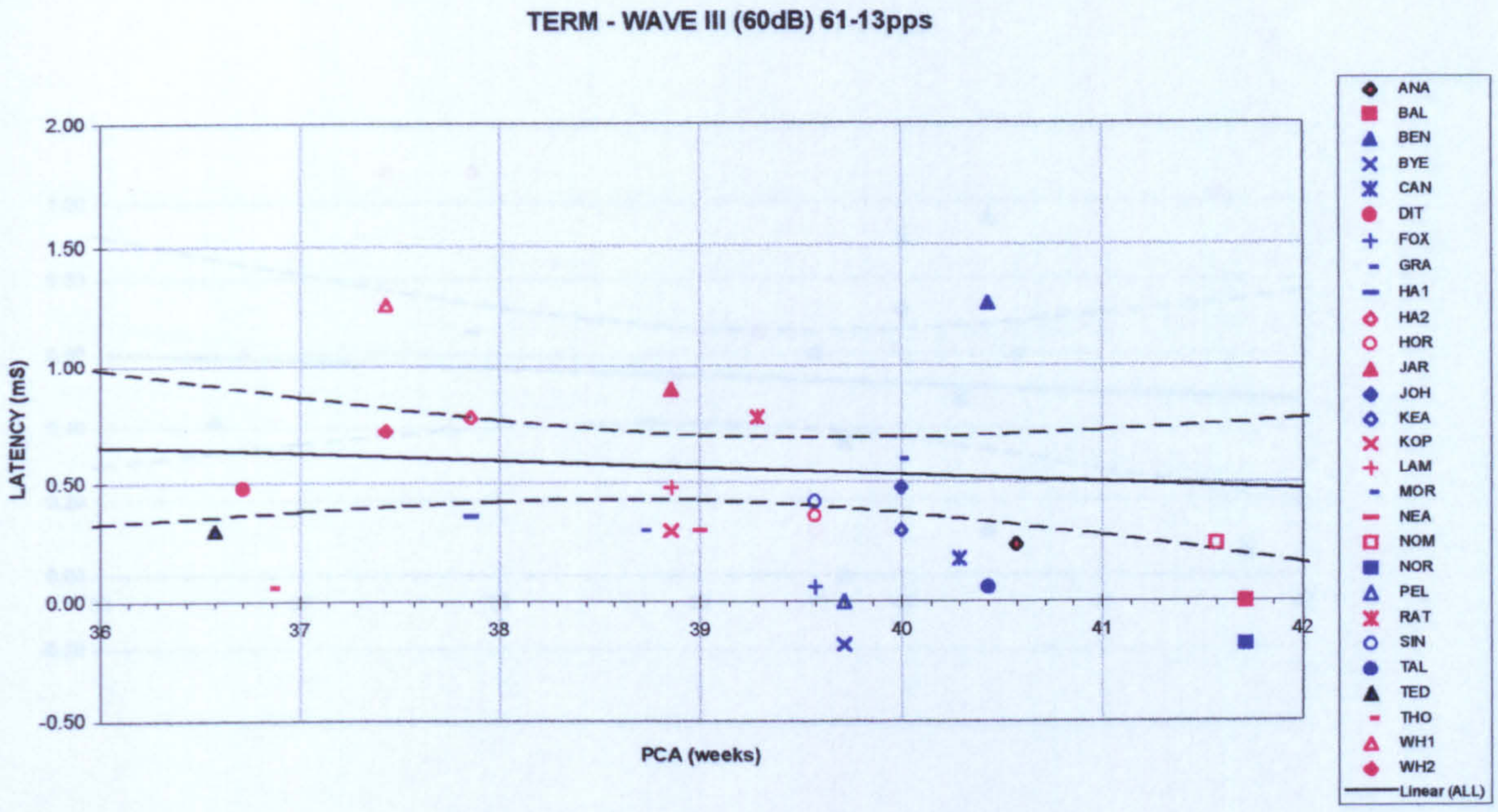


r^2=0.12 n=28 P>0.05

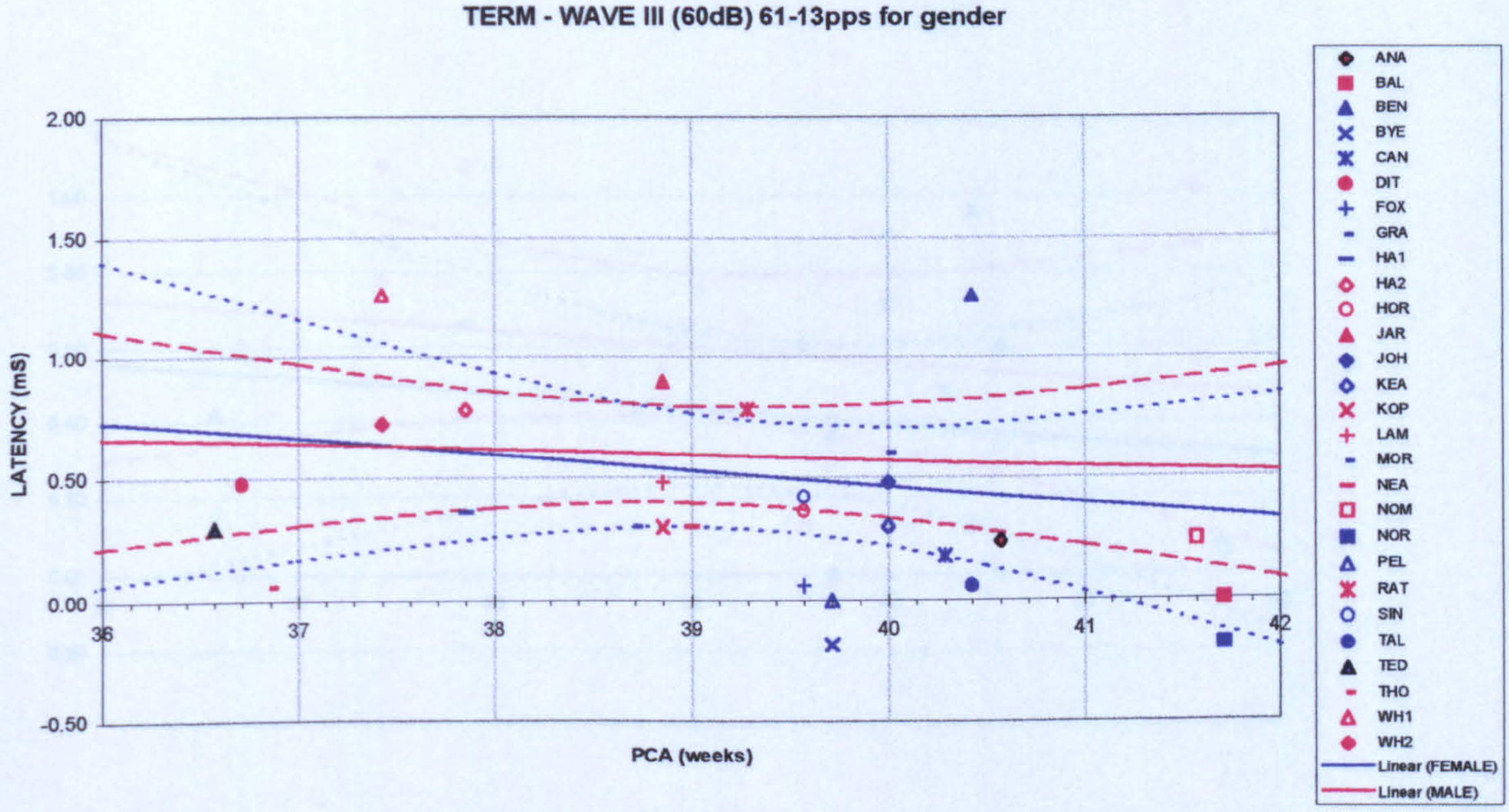


Female - r^2=0.01 n=14 P>0.05 Male - r^2=0.21 n=14 P>0.05

Figure C24 a/b

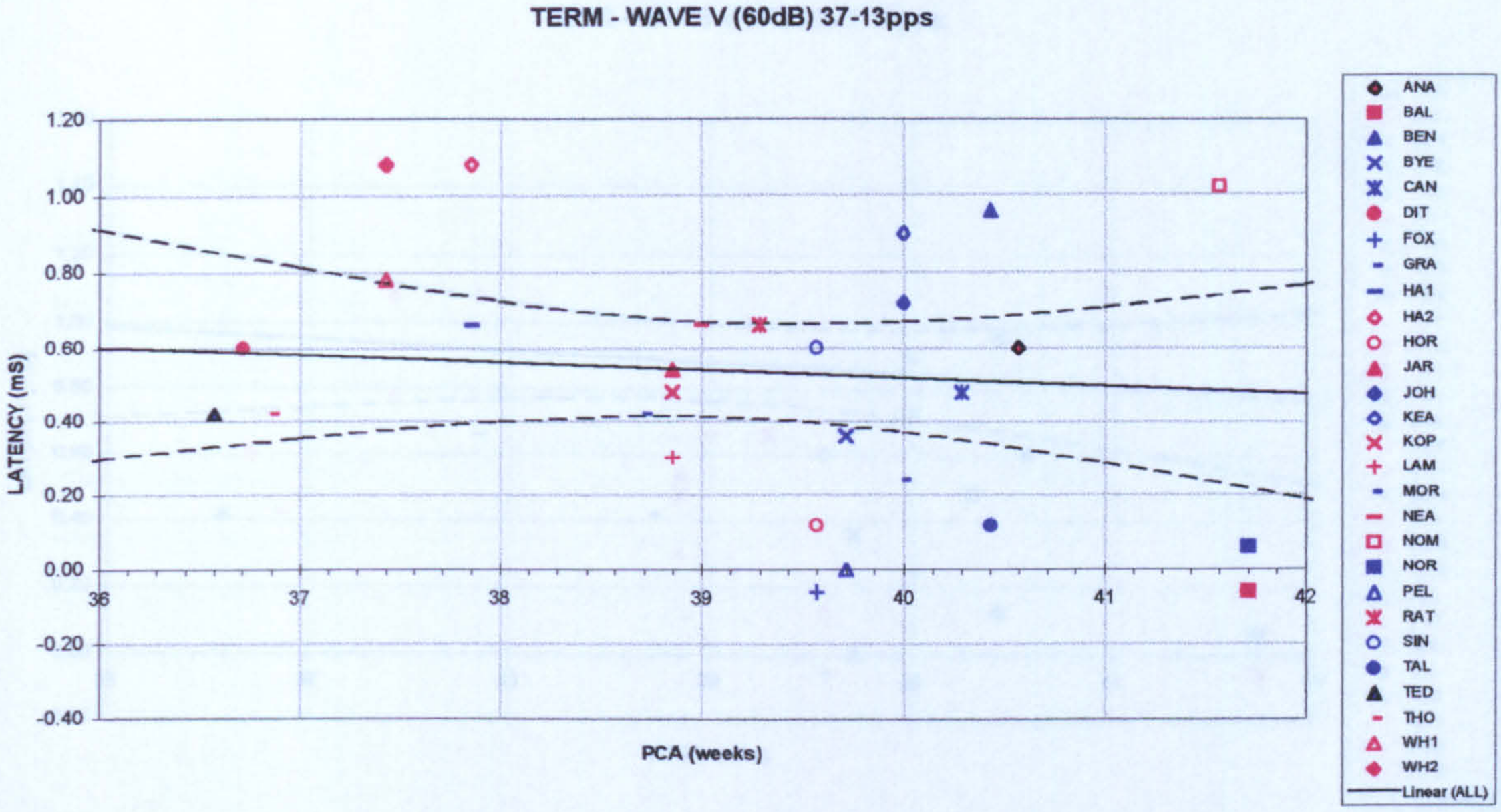


r^2=0.06 n=28 P>0.05

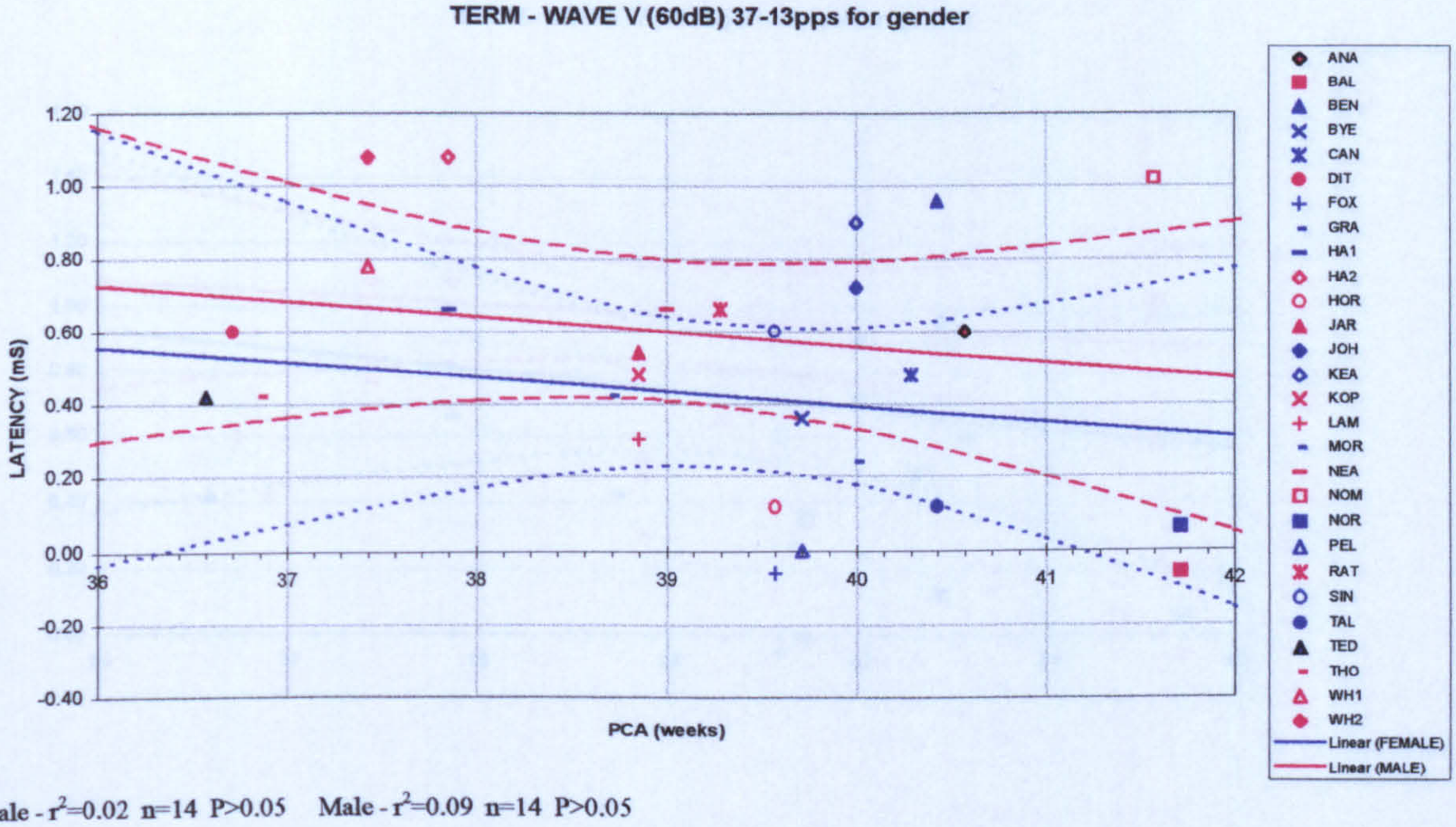


Female - r^2=0.05 n=14 P>0.05 Male - r^2=0.04 n=14 P>0.05

Figure C25 a/b

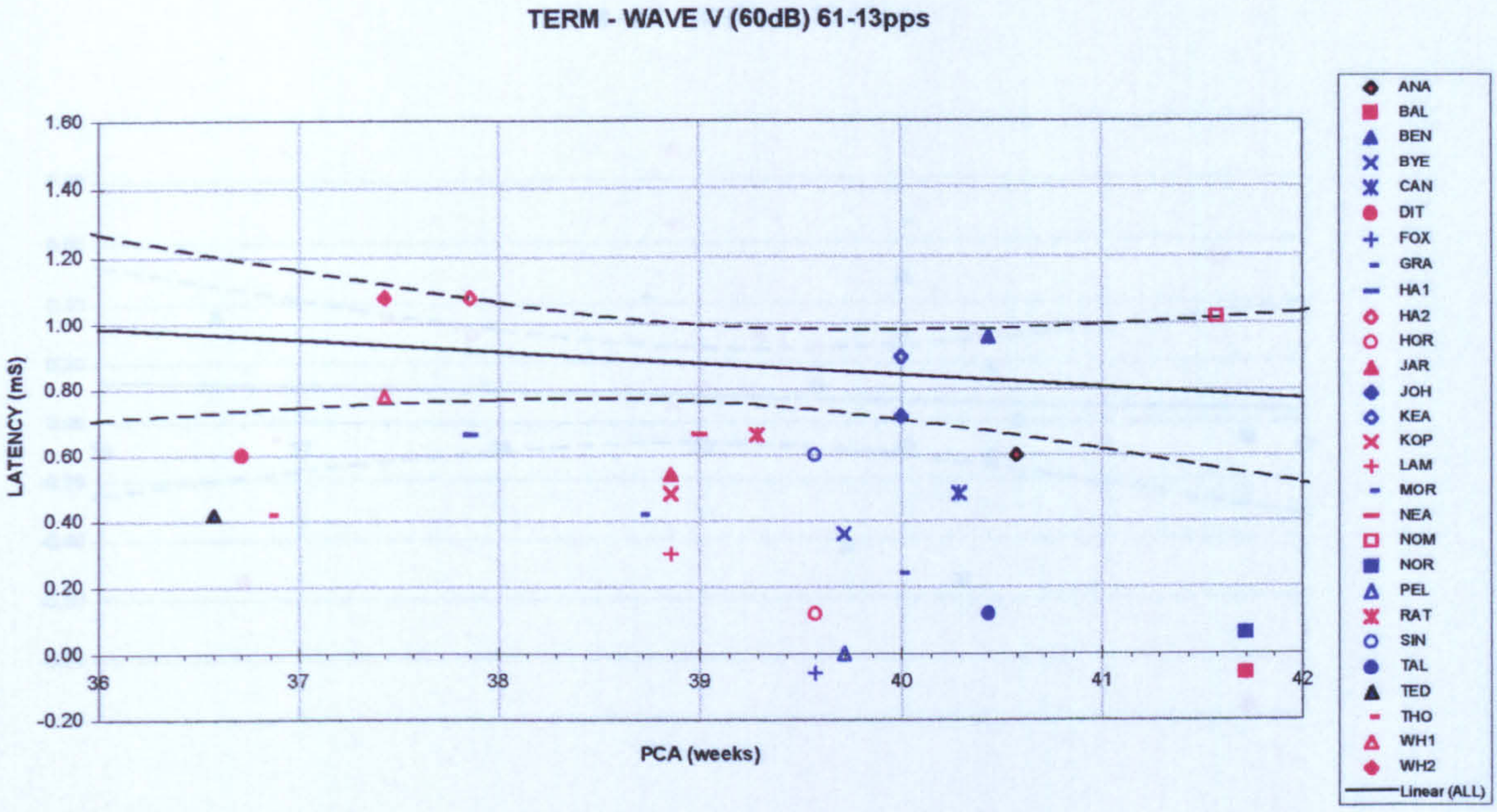


r^2=0.08 n=28 P>0.05

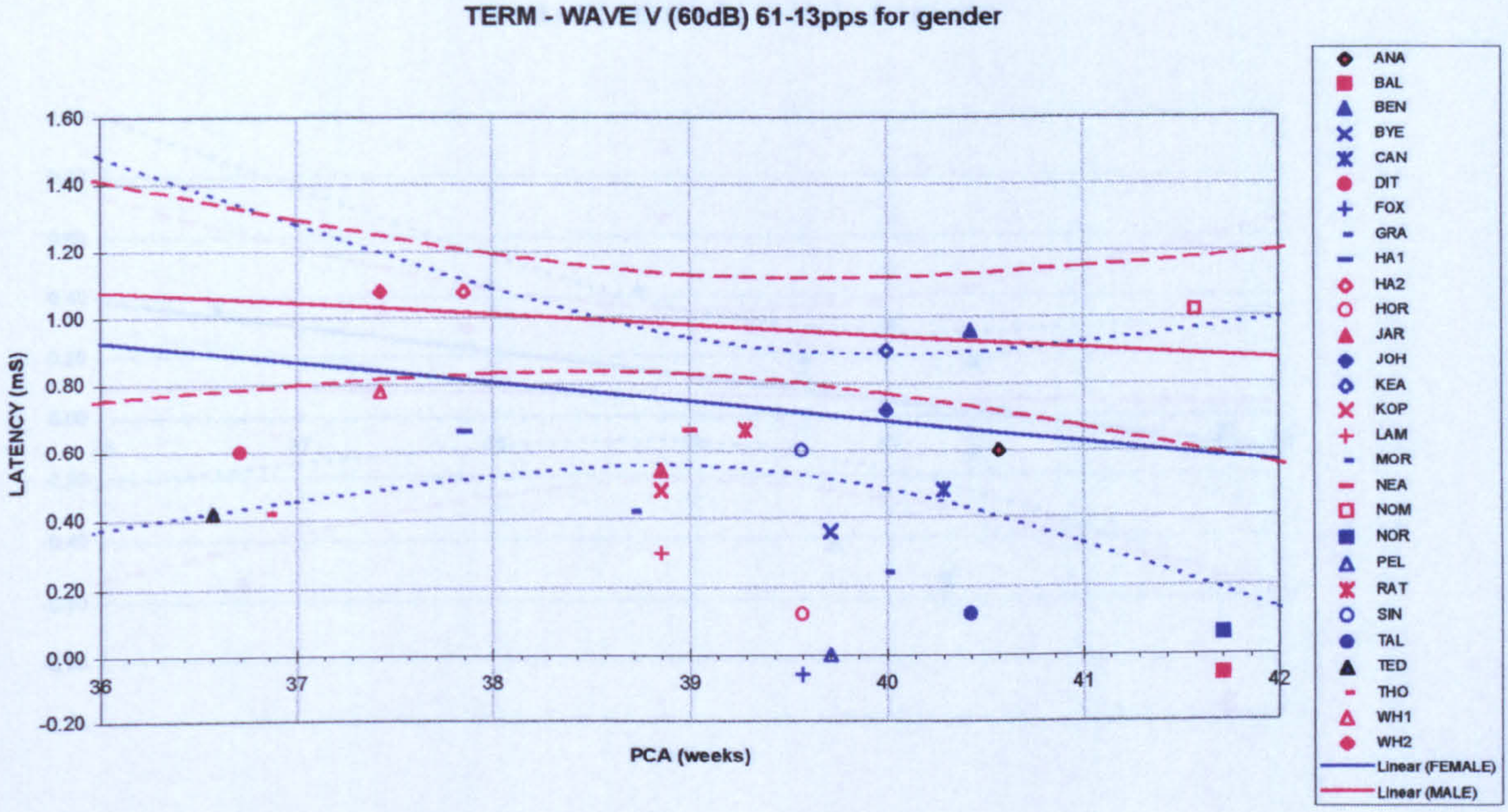


Female - r^2=0.02 n=14 P>0.05 Male - r^2=0.09 n=14 P>0.05

Figure C26 a/b

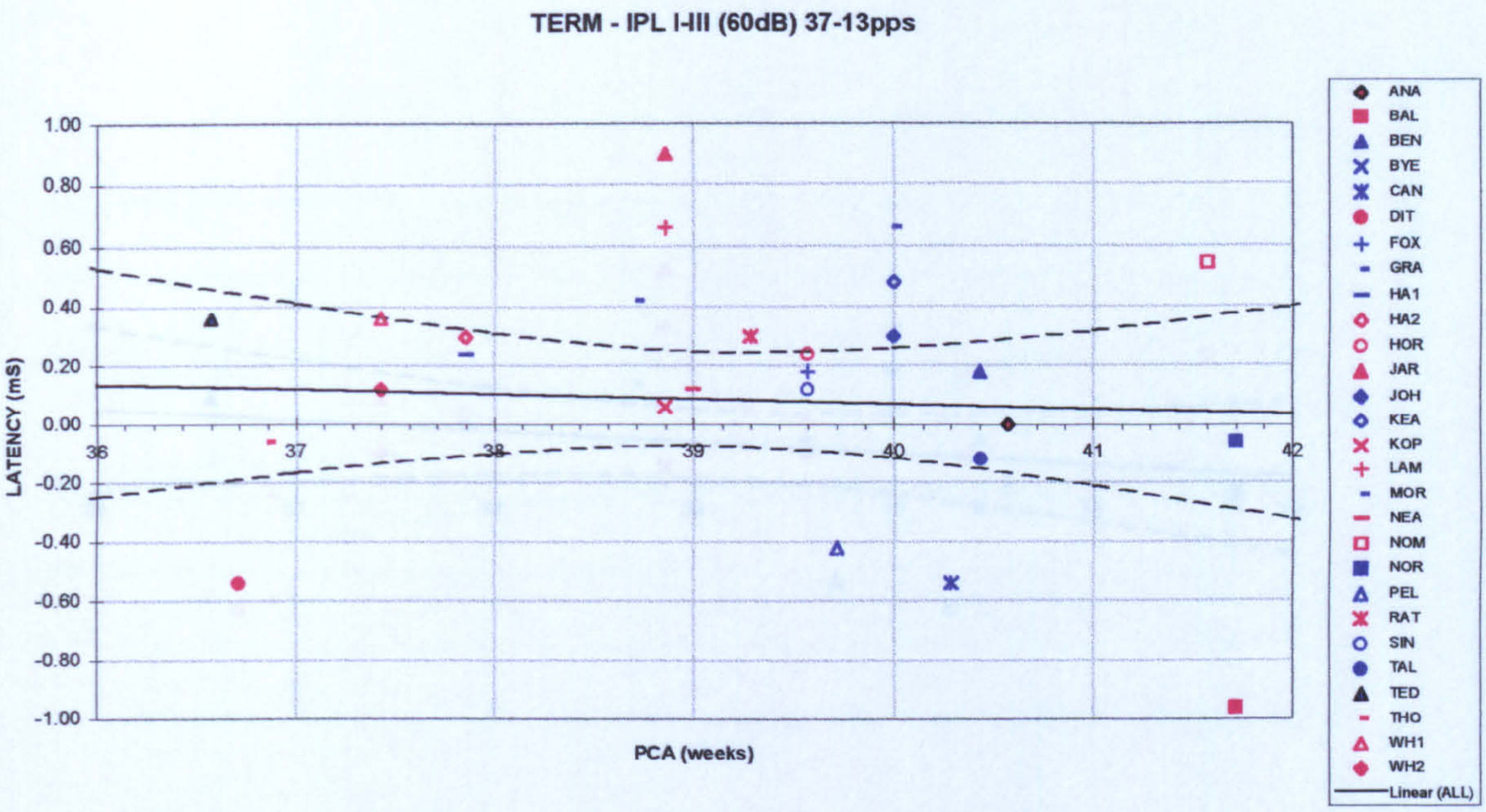


r^2=0.13 n=28 P>0.05

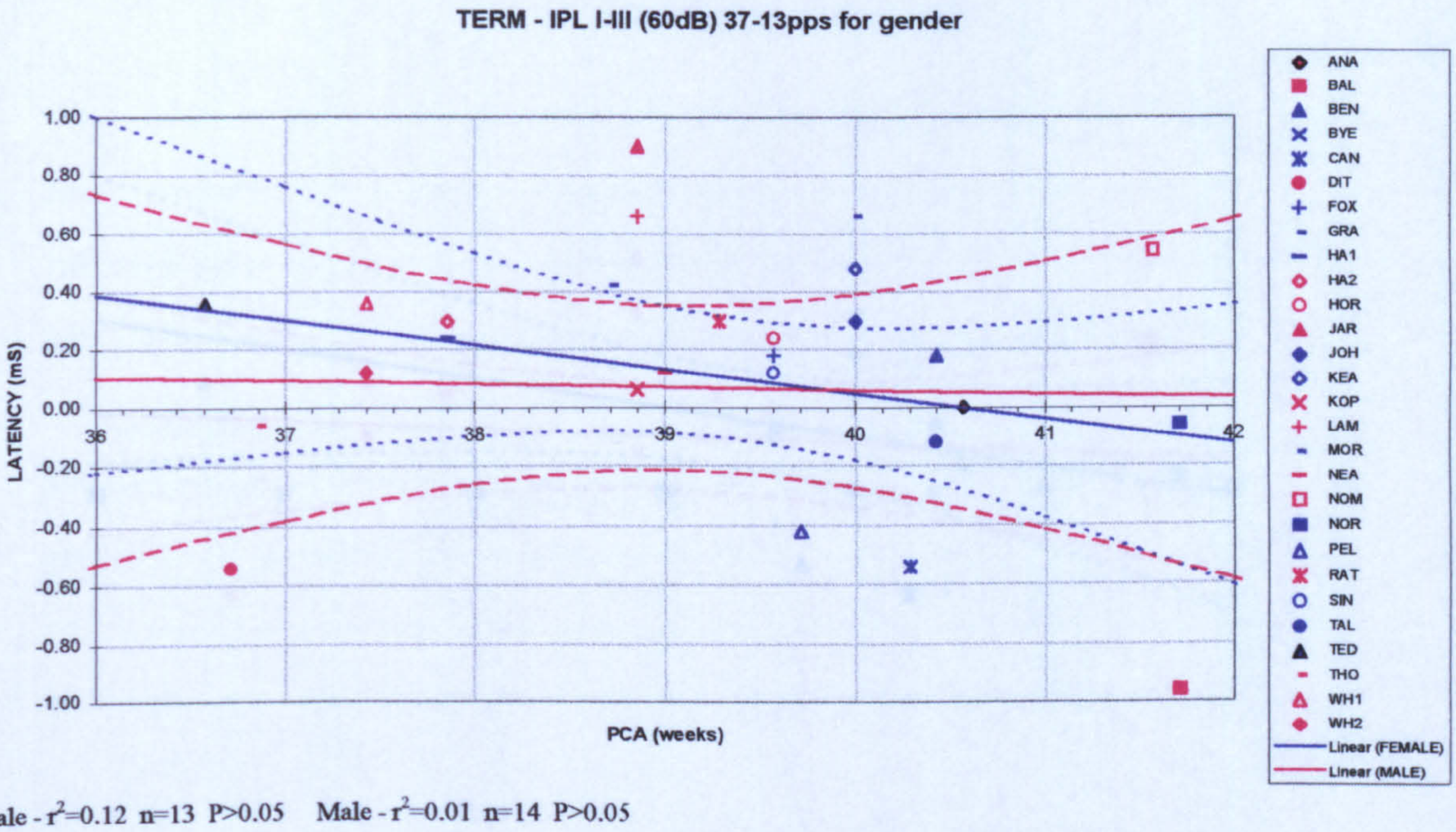


Female - r^2=0.06 n=14 P>0.05 Male - r^2=0.11 n=14 P>0.05

Figure C27 a/b

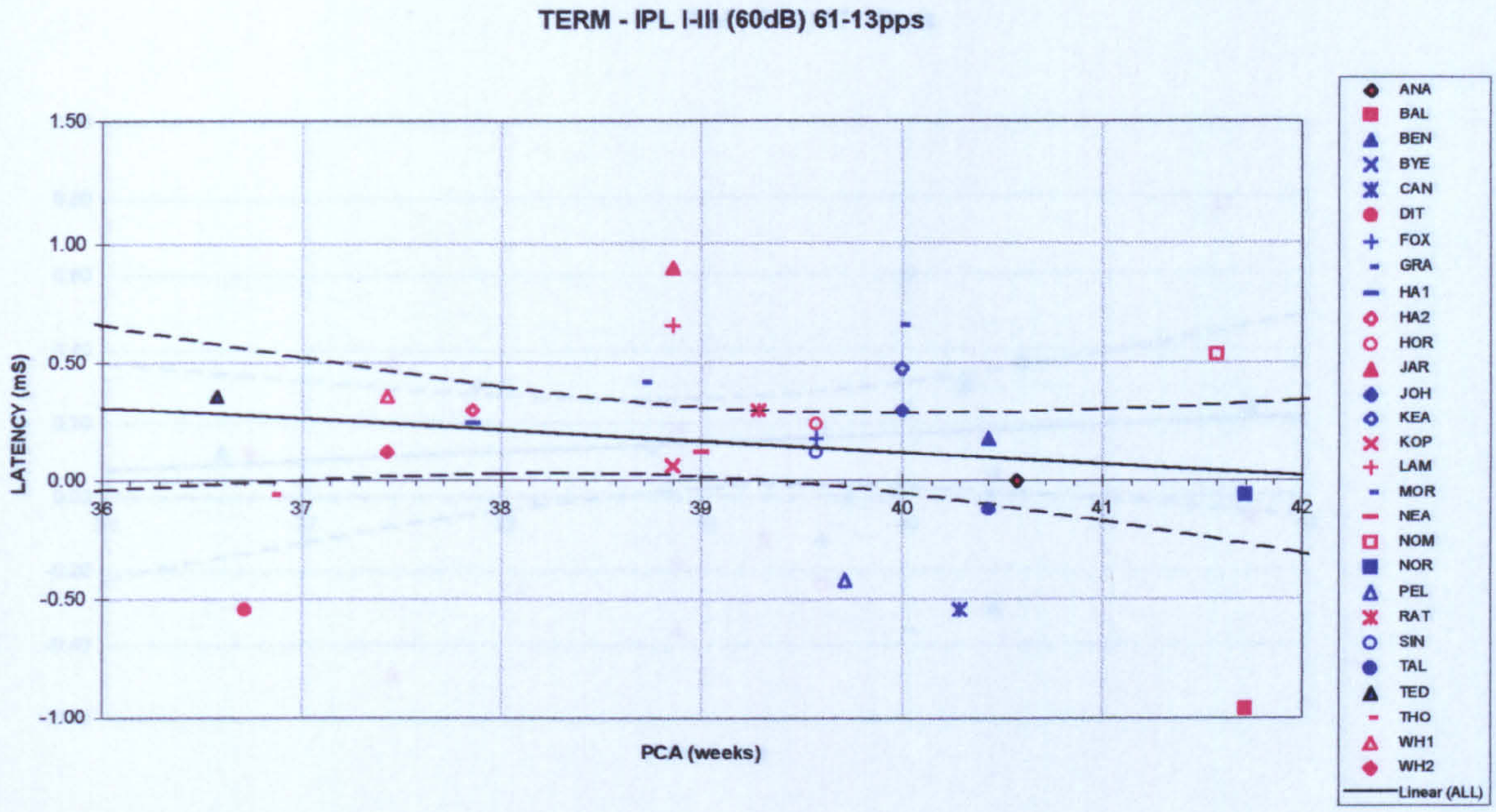


r^2=0.03 n=27 P>0.05

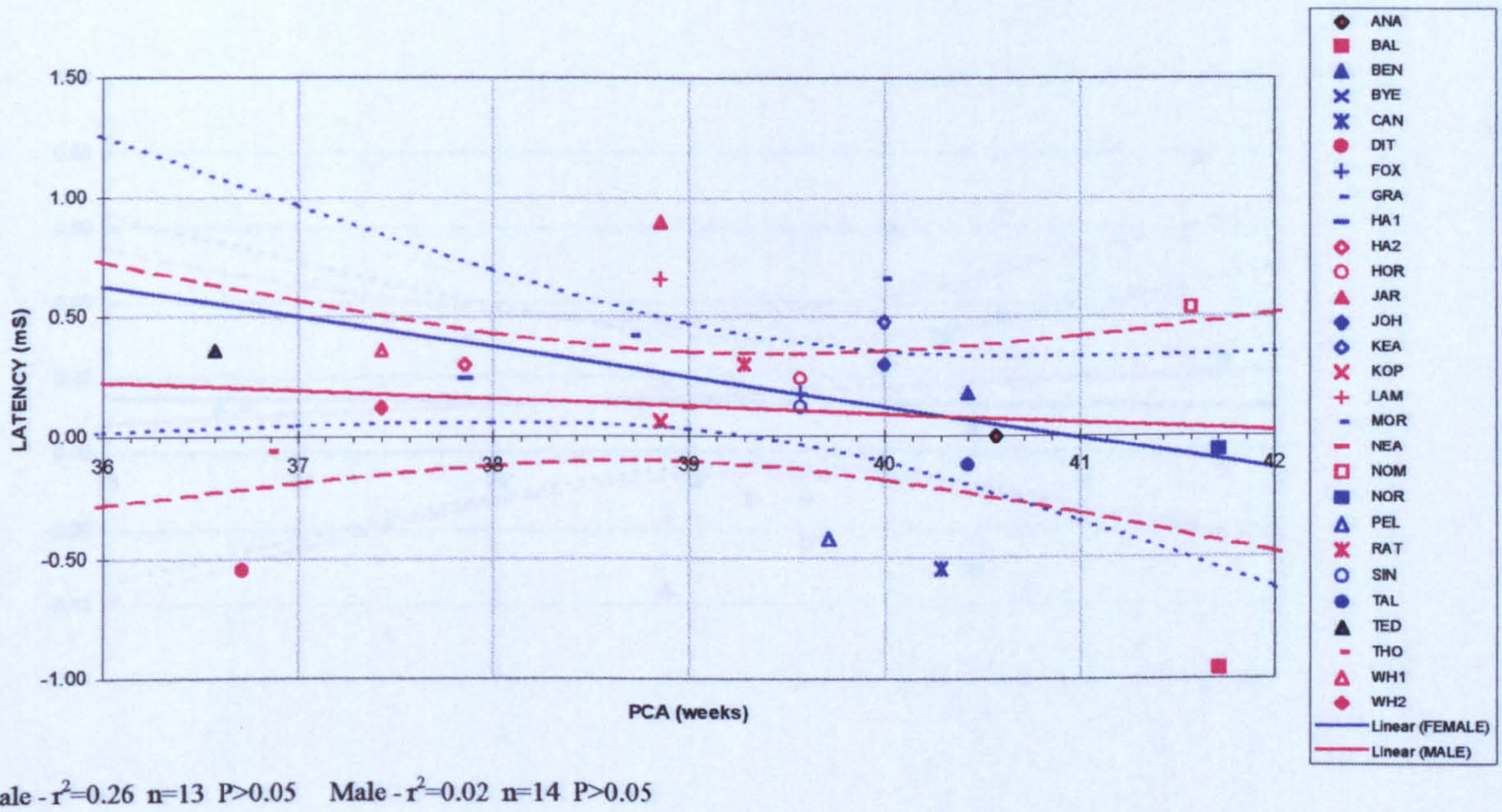


Female - r^2=0.12 n=13 P>0.05 Male - r^2=0.01 n=14 P>0.05

Figure C28 a/b

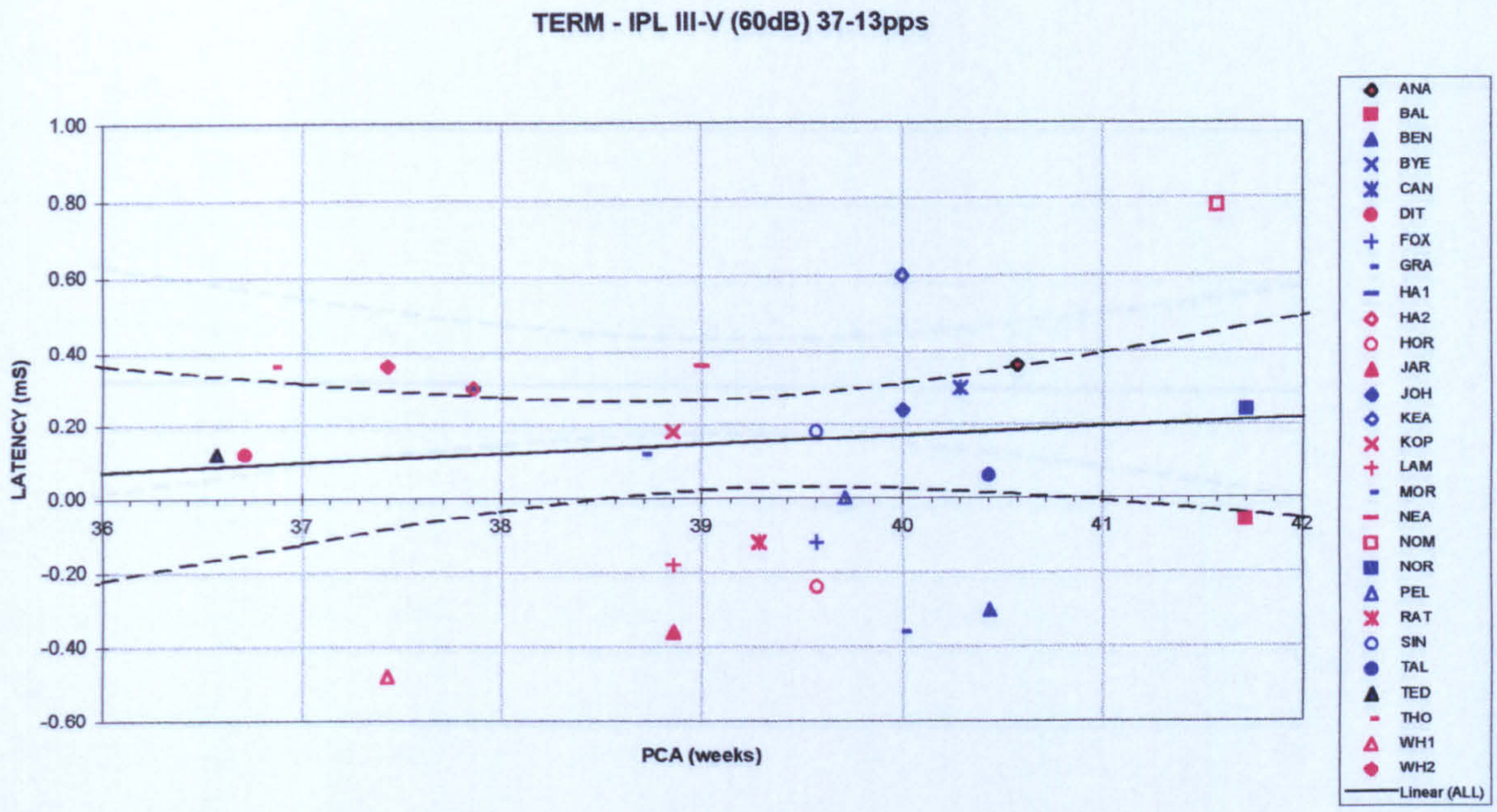


r^2=0.07 n=27 P>0.05

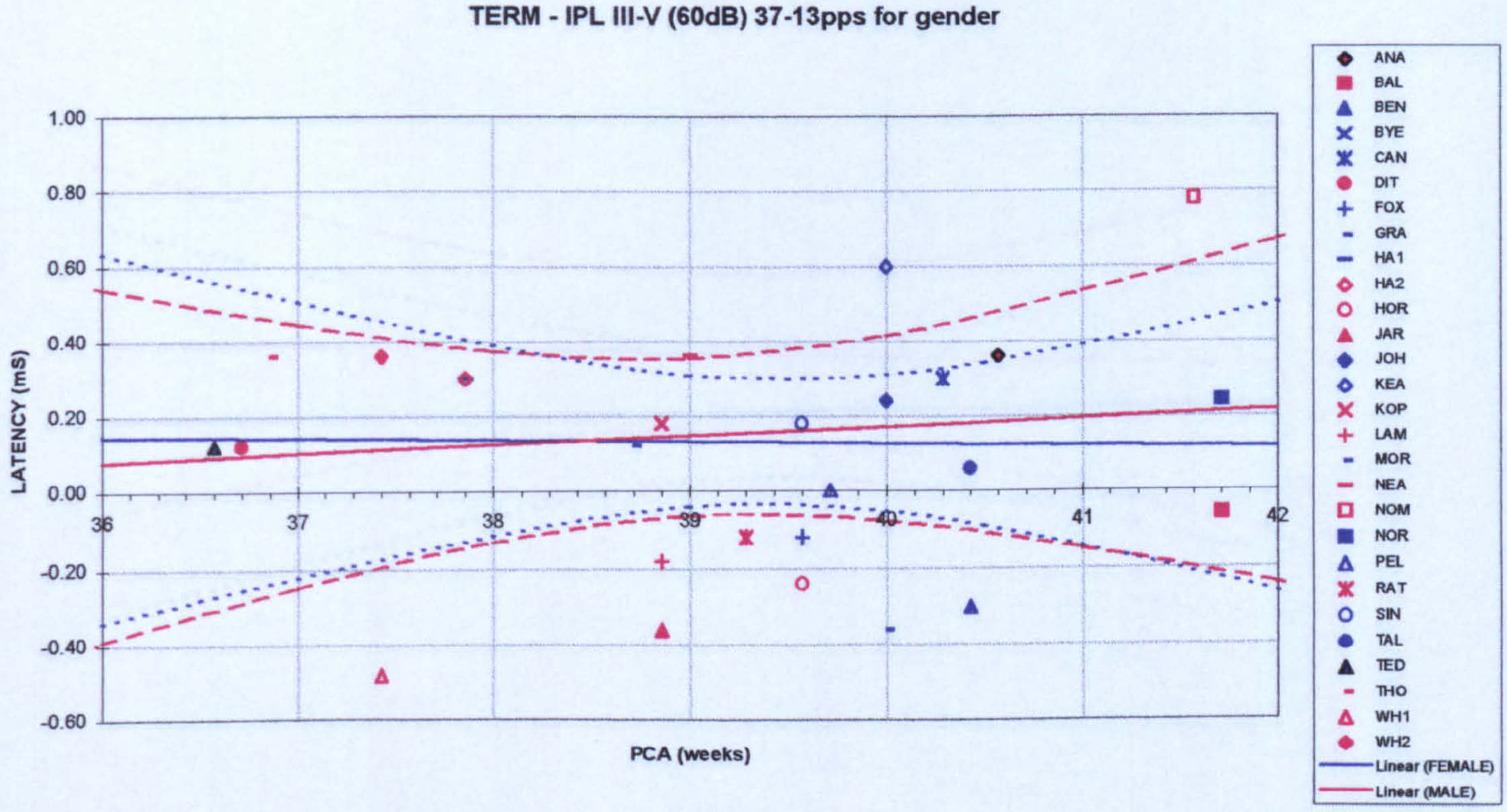


Female - r^2=0.26 n=13 P>0.05 Male - r^2=0.02 n=14 P>0.05

Figure C29 a/b

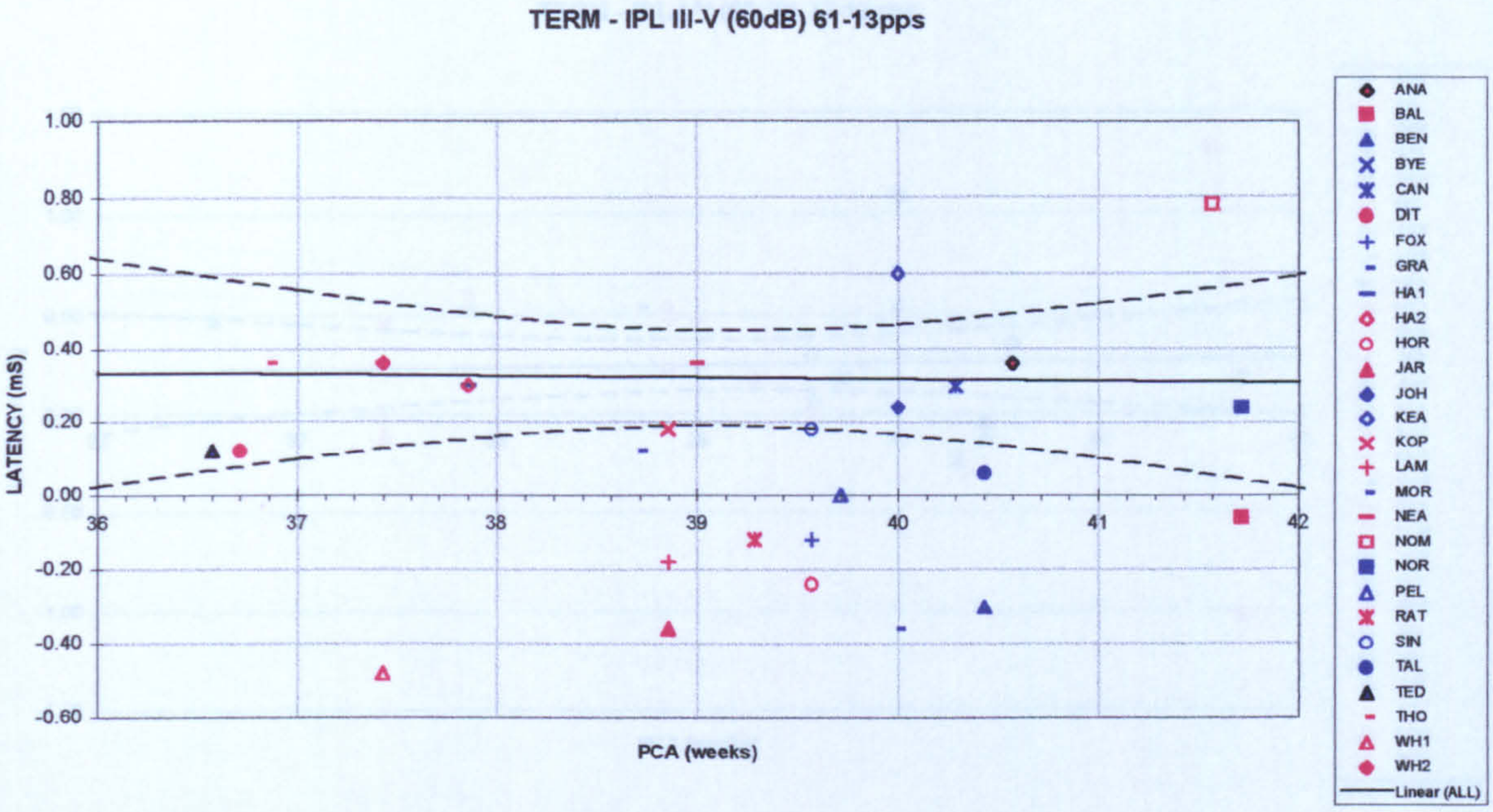


r^2=0.01 n=27 P>0.05

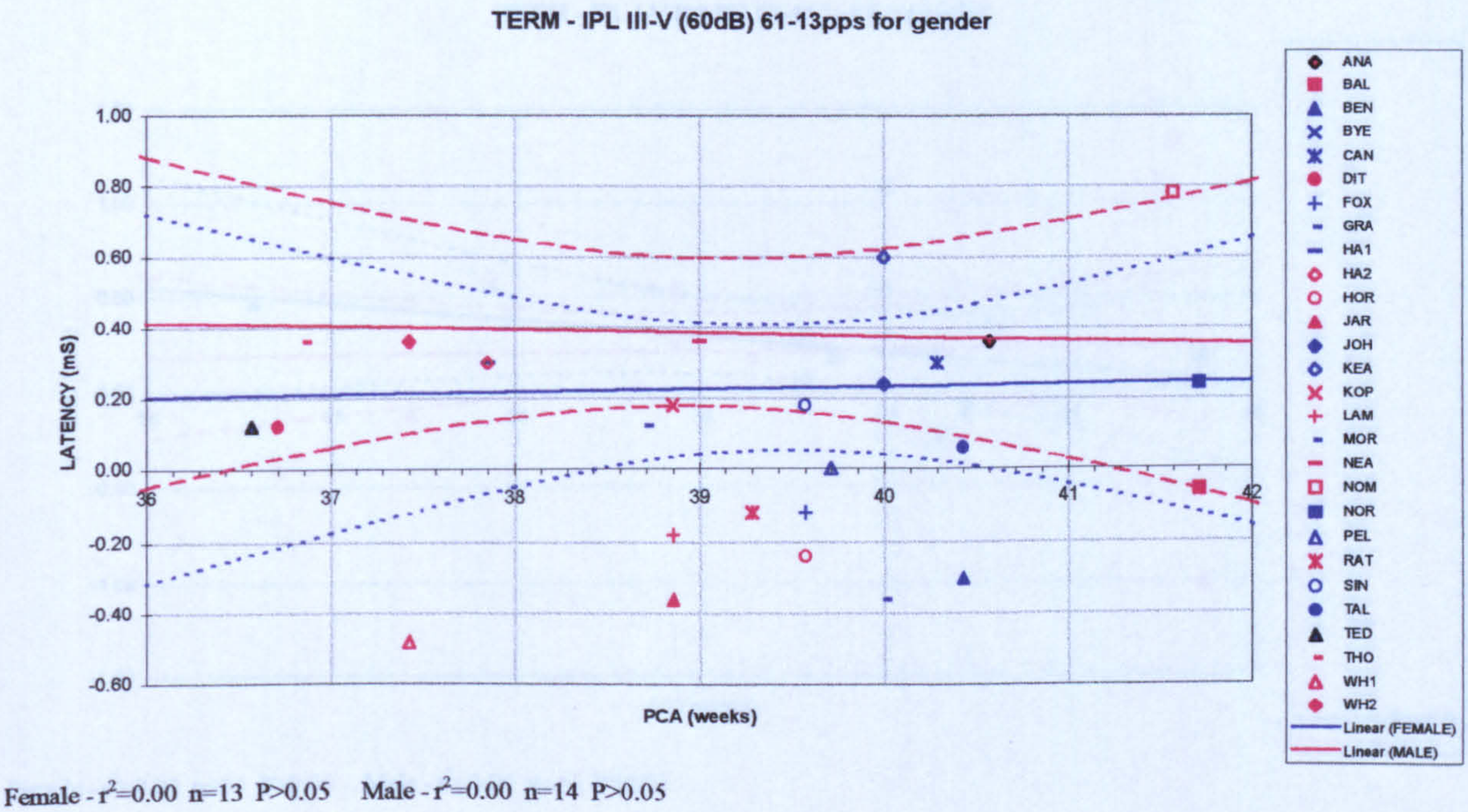


Female - r^2=0.01 n=13 P>0.05 Male - r^2=0.03 n=14 P>0.05

Figure C30 a/b

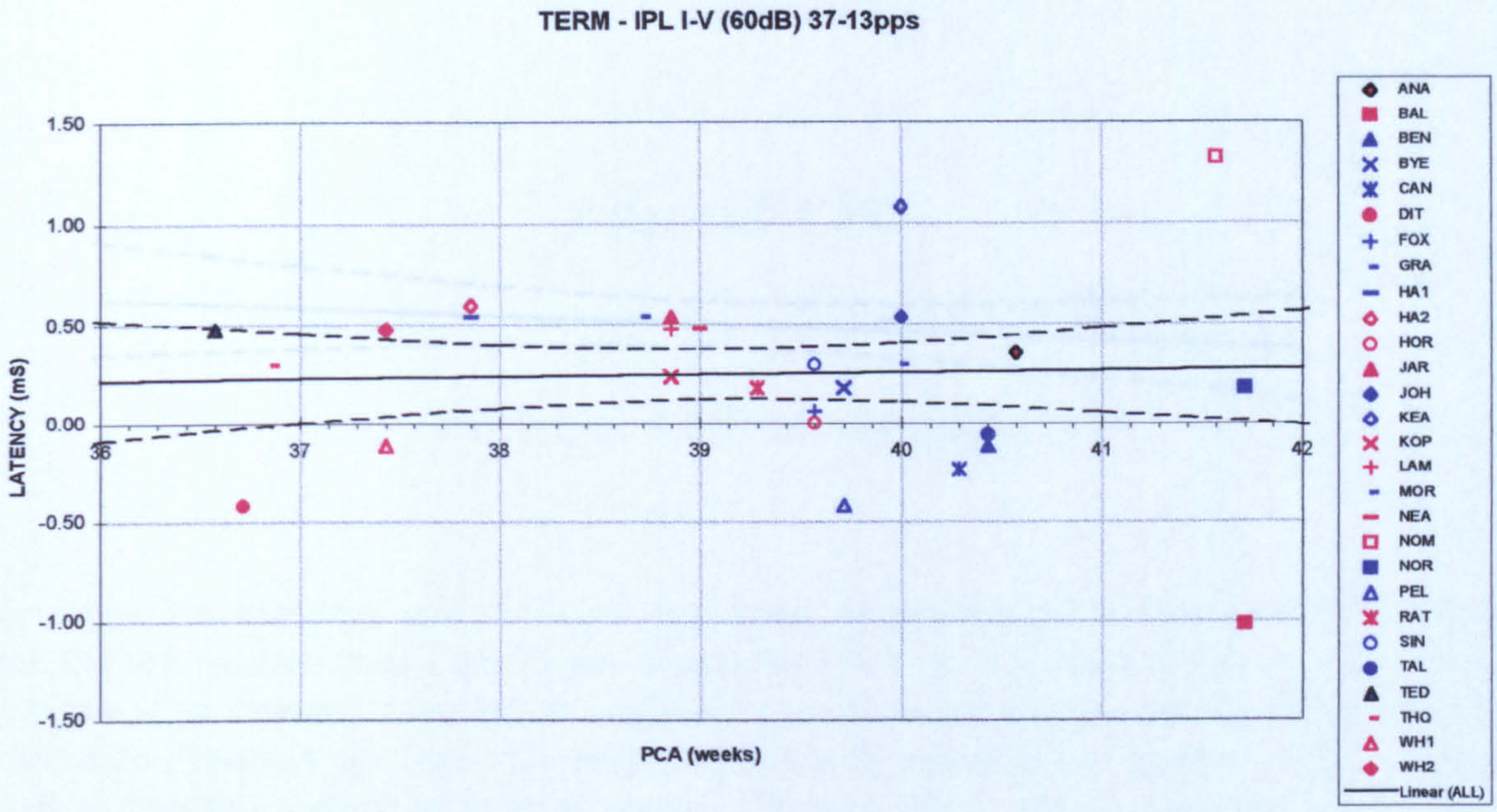


r^2=0.01 n=27 P>0.05

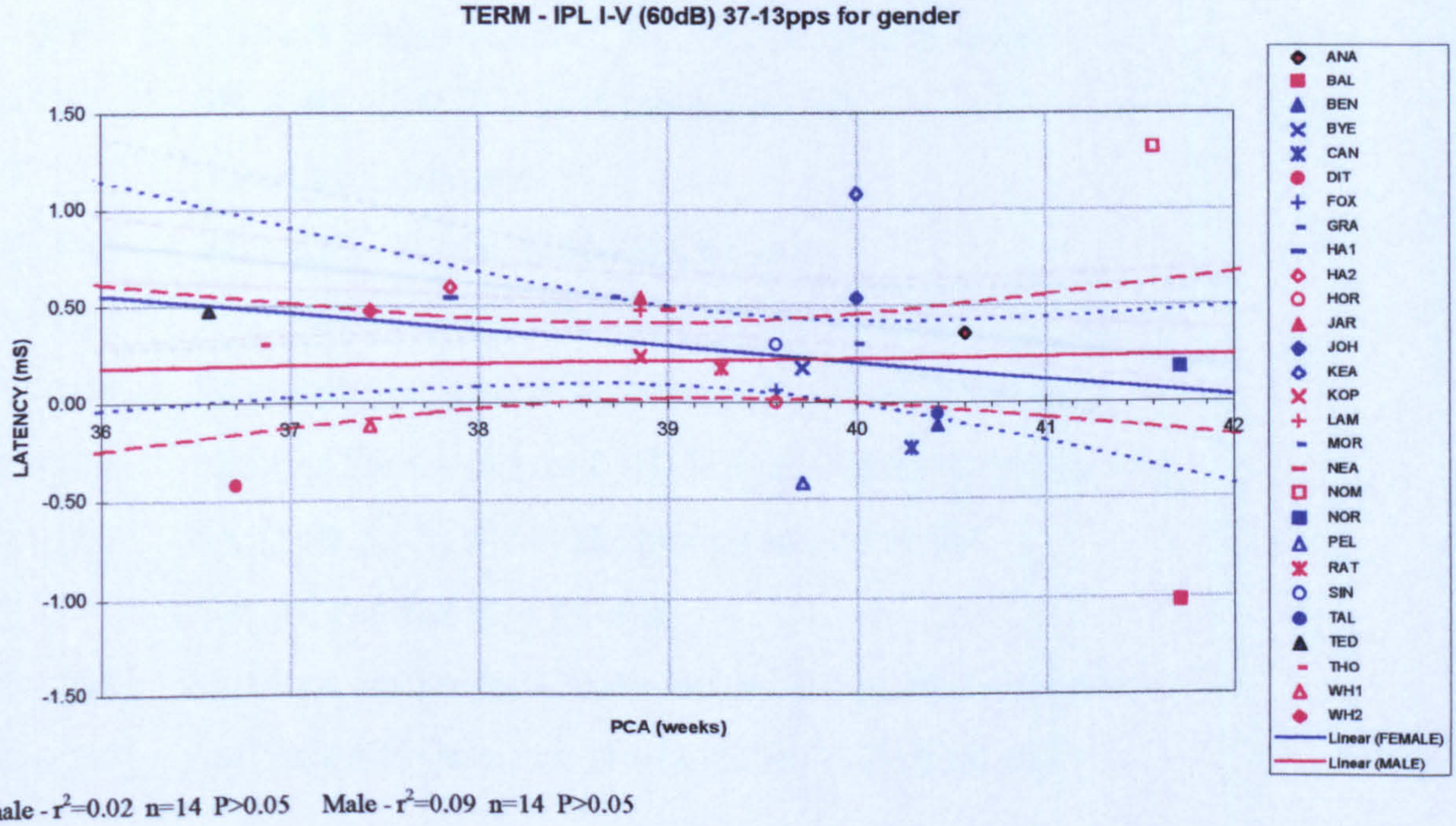


Female - r^2=0.00 n=13 P>0.05 **Male** - r^2=0.00 n=14 P>0.05

Figure C31 a/b

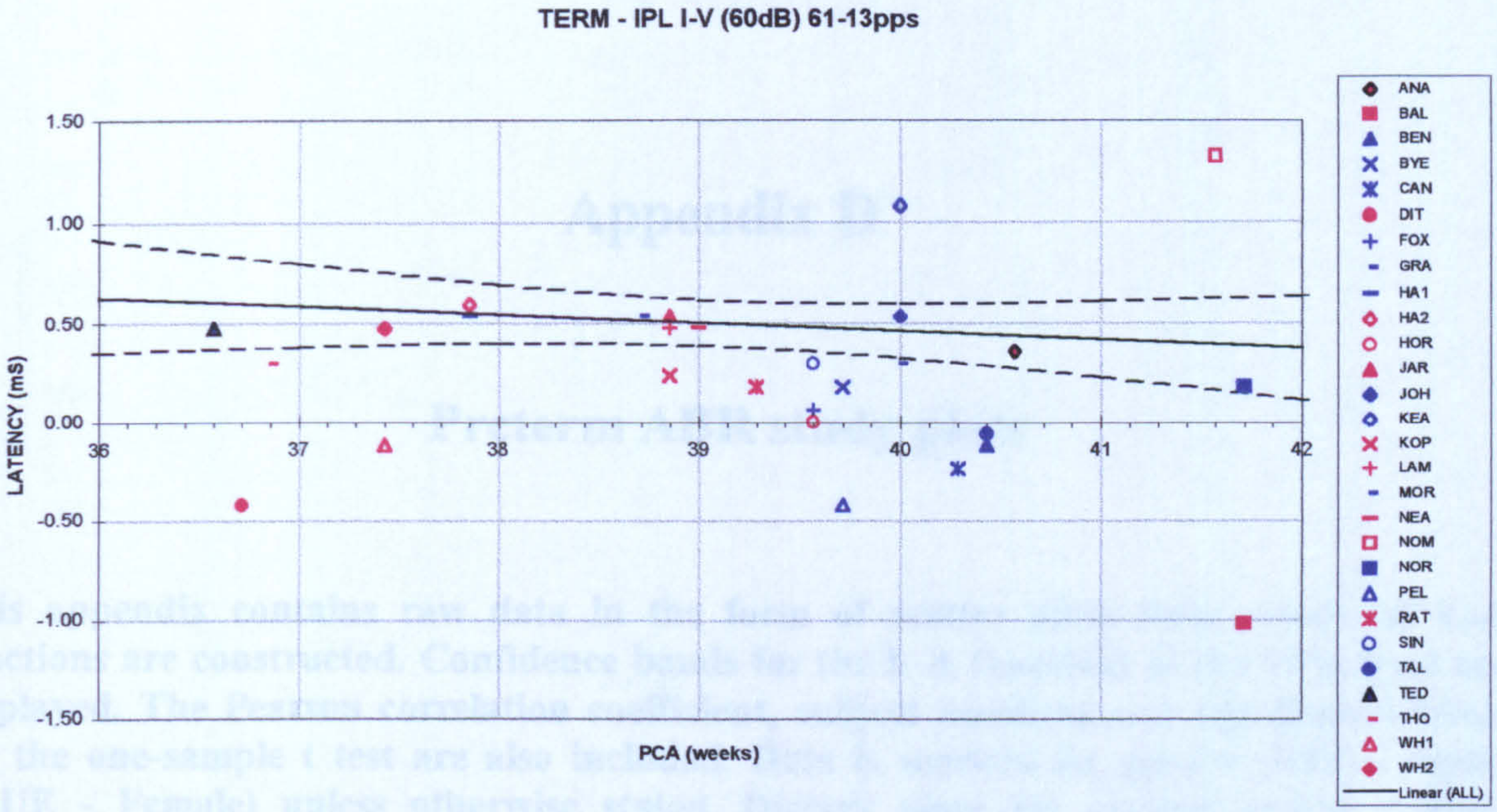


r^2=0.08 n=28 P>0.05

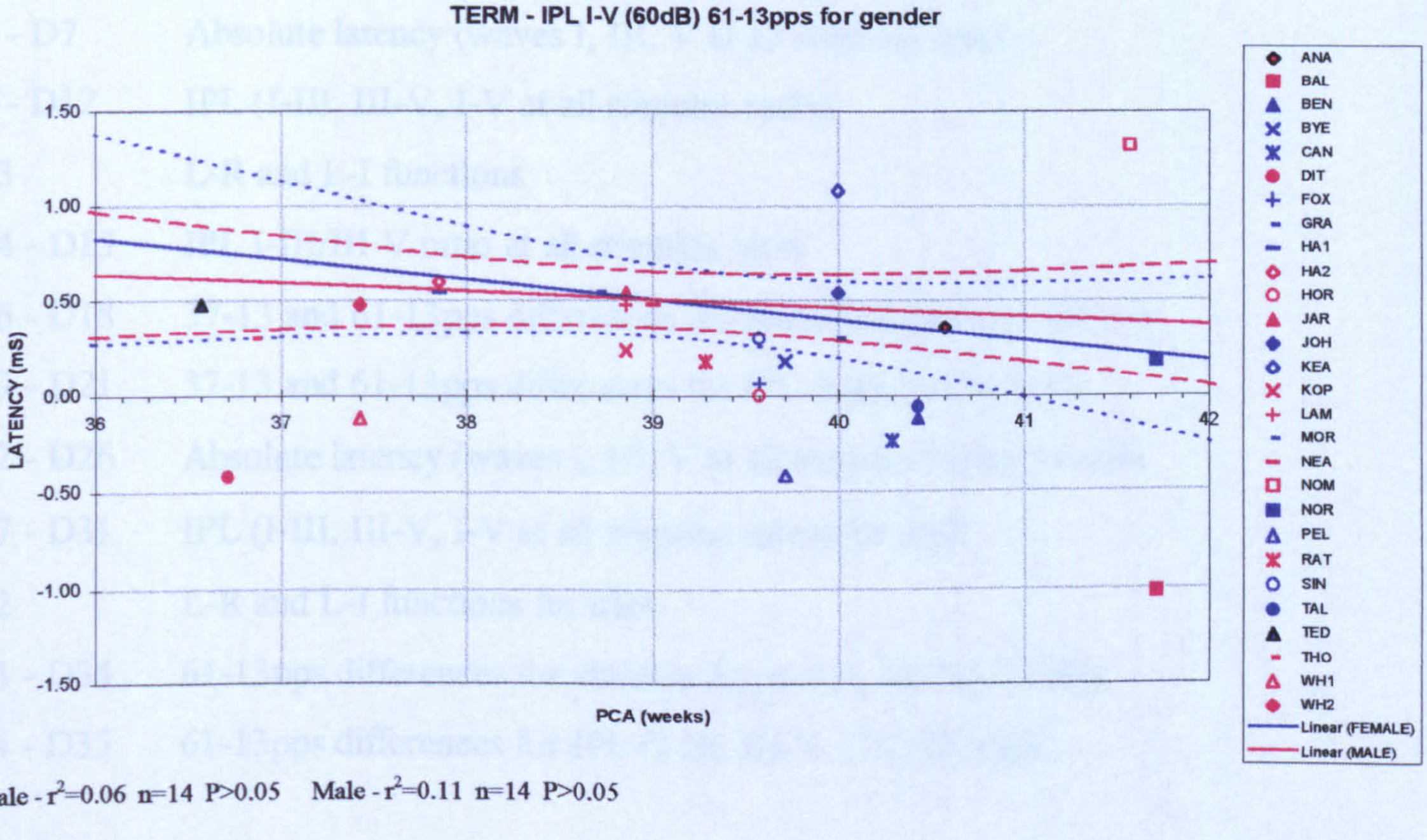


Female - r^2=0.02 n=14 P>0.05 Male - r^2=0.09 n=14 P>0.05

Figure C32 a/b



r^2=0.13 n=28 P>0.05



Female - r^2=0.06 n=14 P>0.05 Male - r^2=0.11 n=14 P>0.05

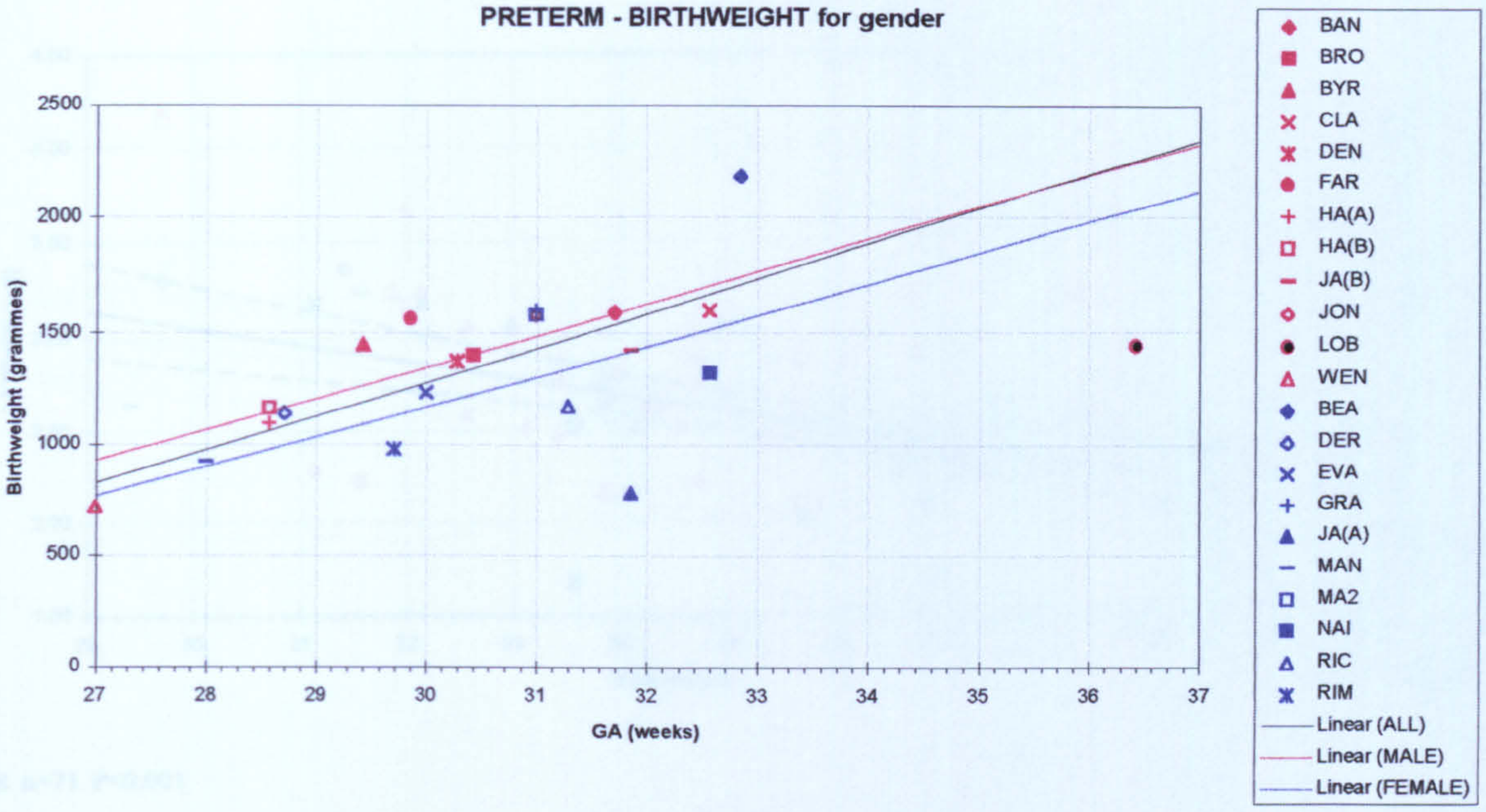
Appendix D

Preterm ABR study plots

This appendix contains raw data in the form of scatter plots from which all L-A functions are constructed. Confidence bands for the L-A functions at the 95% level are displayed. The Pearson correlation coefficient, subject numbers and significance levels for the one-sample t test are also included. Data is marked for gender (RED - Male, BLUE - Female) unless otherwise stated. Dietary plots are marked (EBM - Blue, Prematil - Red, C&G - Green).

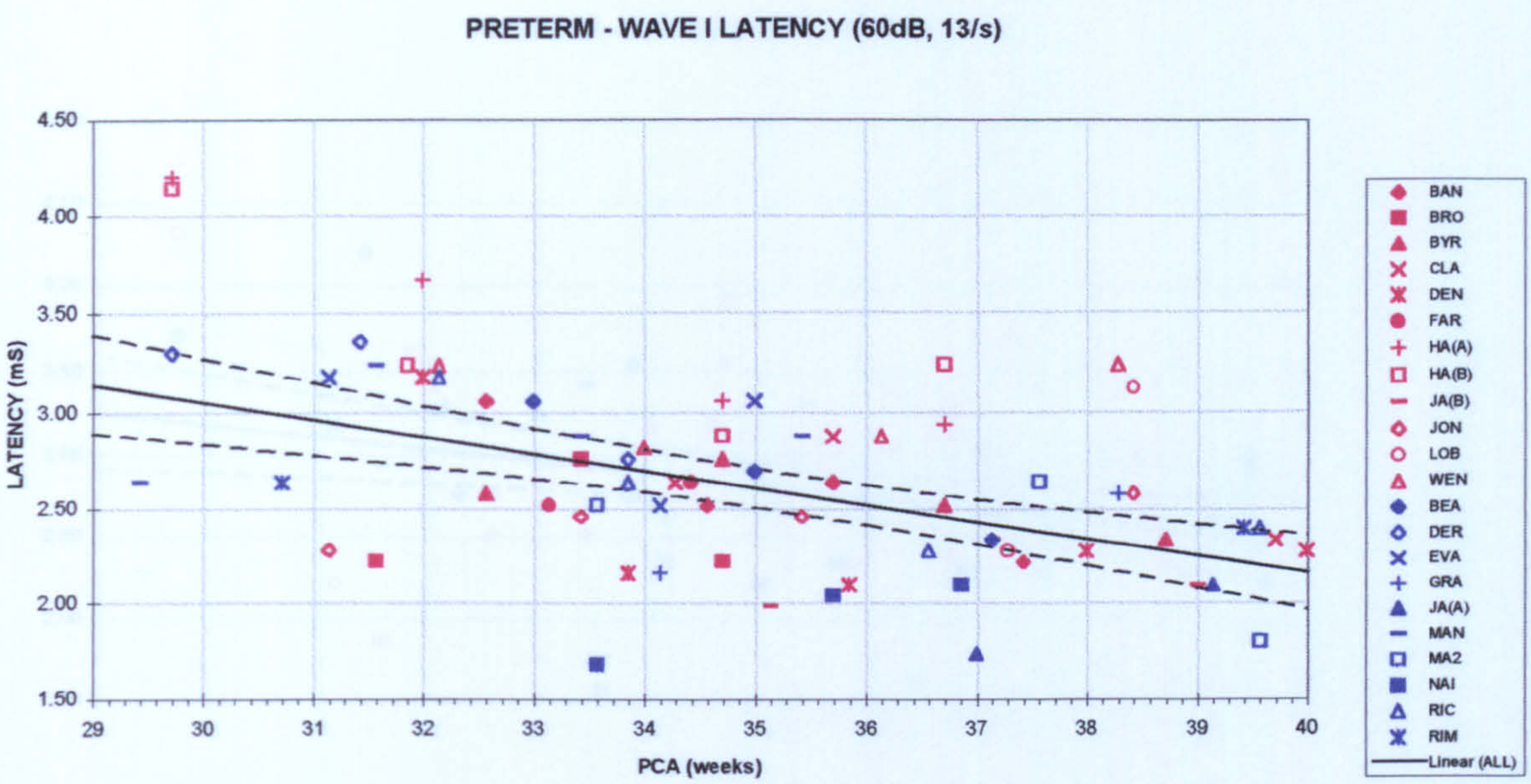
D2	GA versus birthweight
D3 - D7	Absolute latency (waves I, III, V at all stimulus rates)
D8 - D12	IPL (I-III, III-V, I-V at all stimulus rates)
D13	L-R and L-I functions
D14 - D15	IPL I-III/III-V ratio at all stimulus rates
D16 - D18	37-13 and 61-13pps differences for absolute latency (I, III, V)
D19 - D21	37-13 and 61-13pps differences for IPL (I-III, III-V, I-V)
D22 - D26	Absolute latency (waves I, III, V at all stimulus rates) for **diet**
D27 - D31	IPL (I-III, III-V, I-V at all stimulus rates) for **diet**
D32	L-R and L-I functions for **diet**
D33 - D34	61-13pps differences for absolute latency (I, III, V) for **diet**
D34 - D35	61-13pps differences for IPL (I-III, III-V, I-V) for **diet**

Figure D0

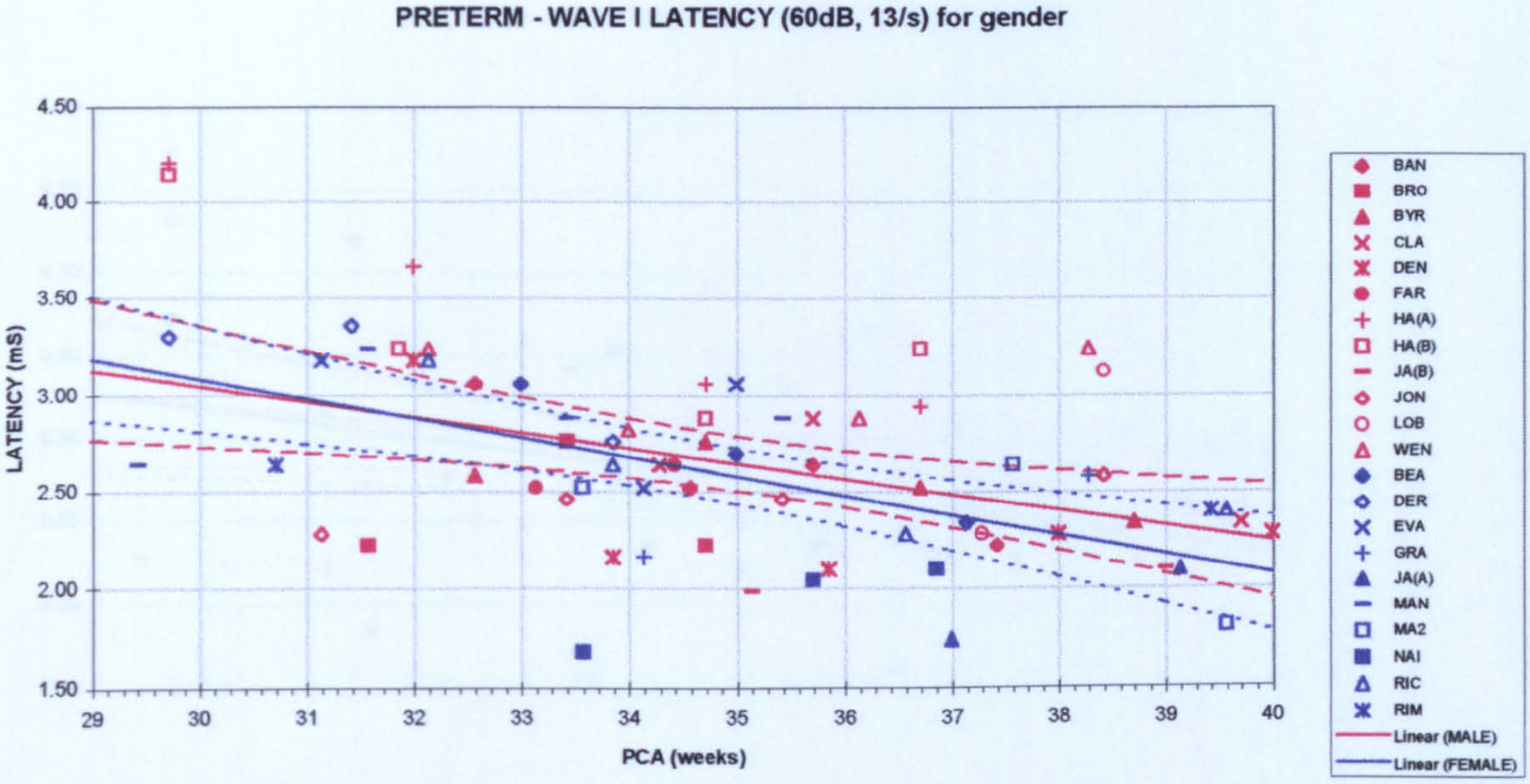


Subject LOB is not included in the linear regression functions.

Figure D1 a/b

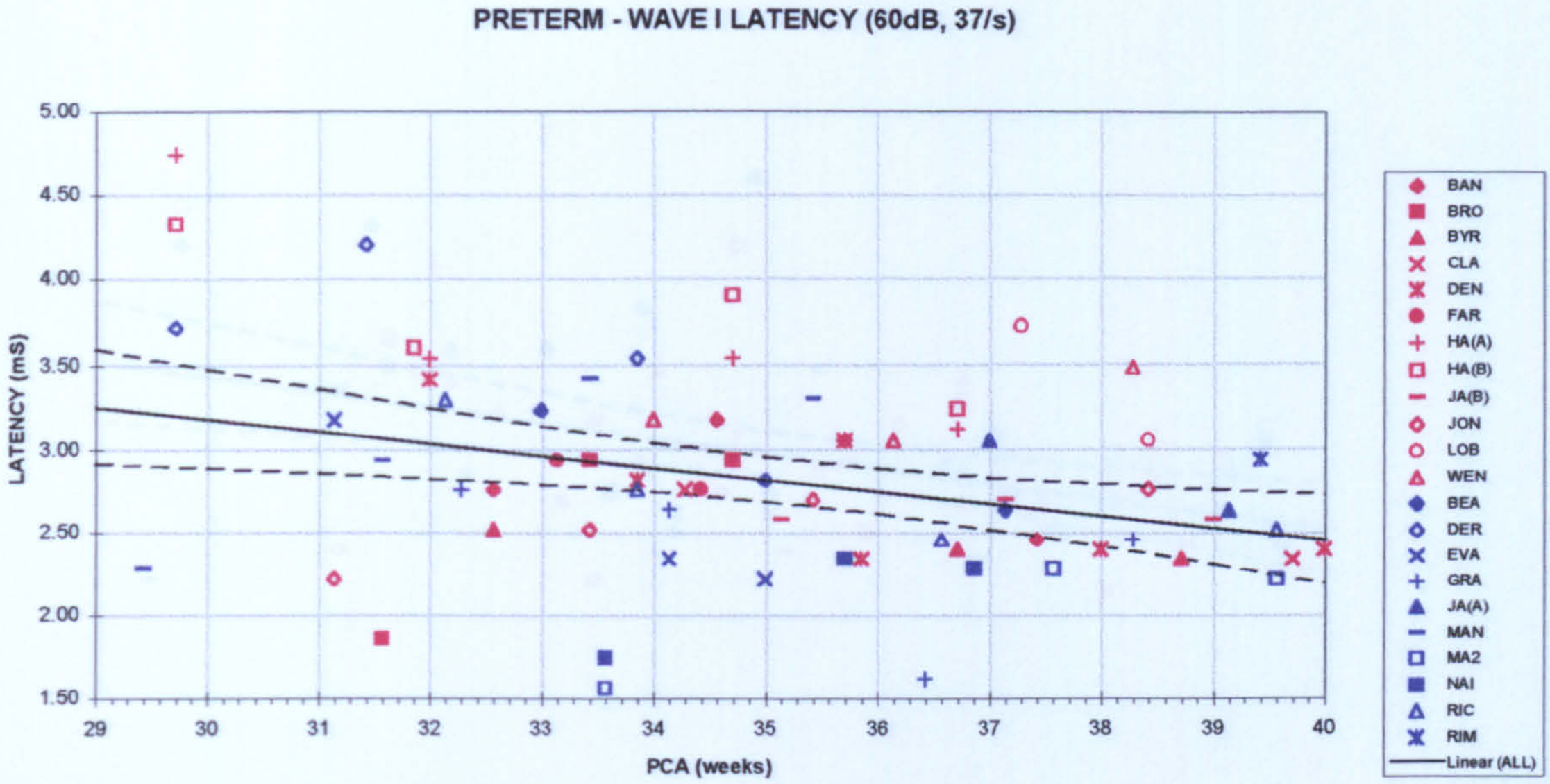


r^2=0.28 n=71 P<0.001

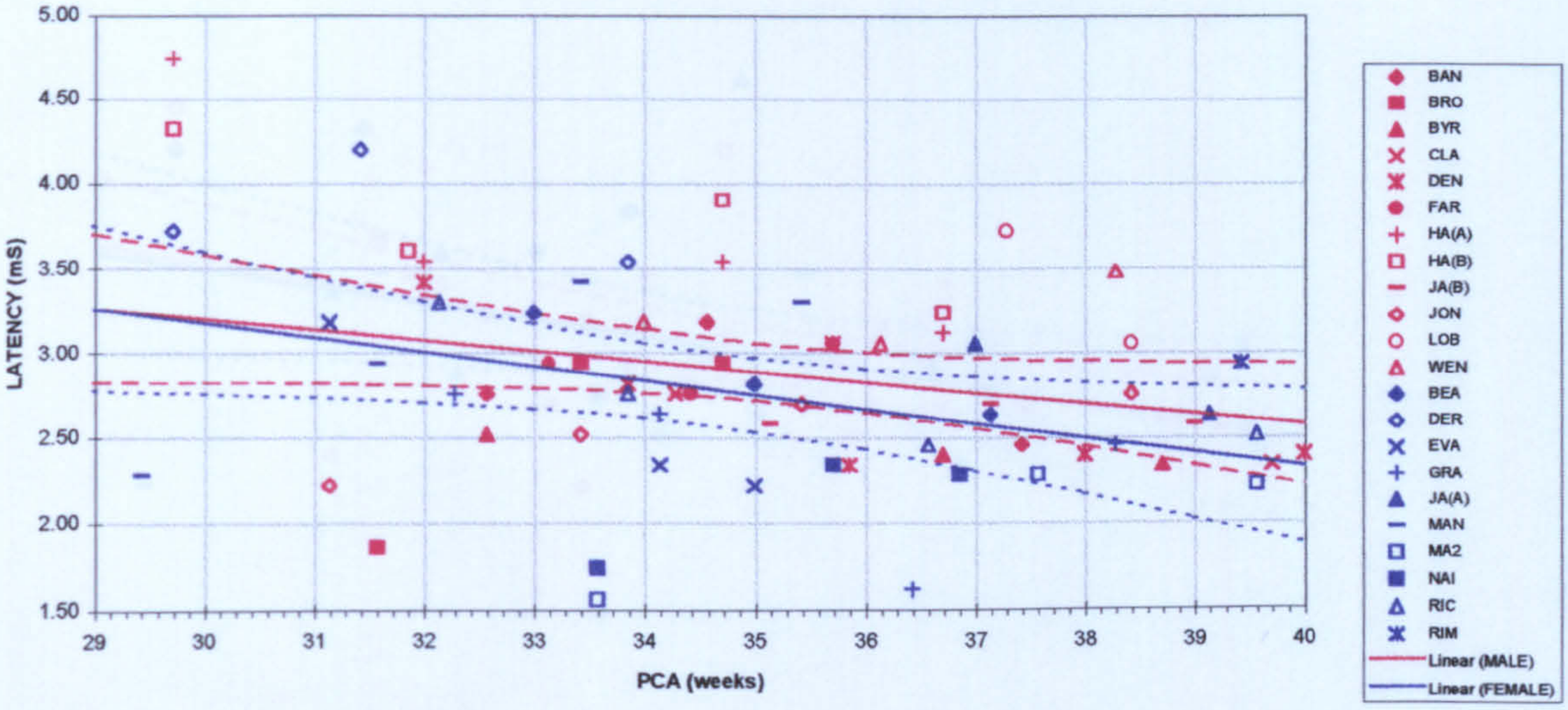


Female - r^2=0.38 n=29 P<0.001 Male - r^2=0.24 n=42 P<0.001

Figure D2 a/b

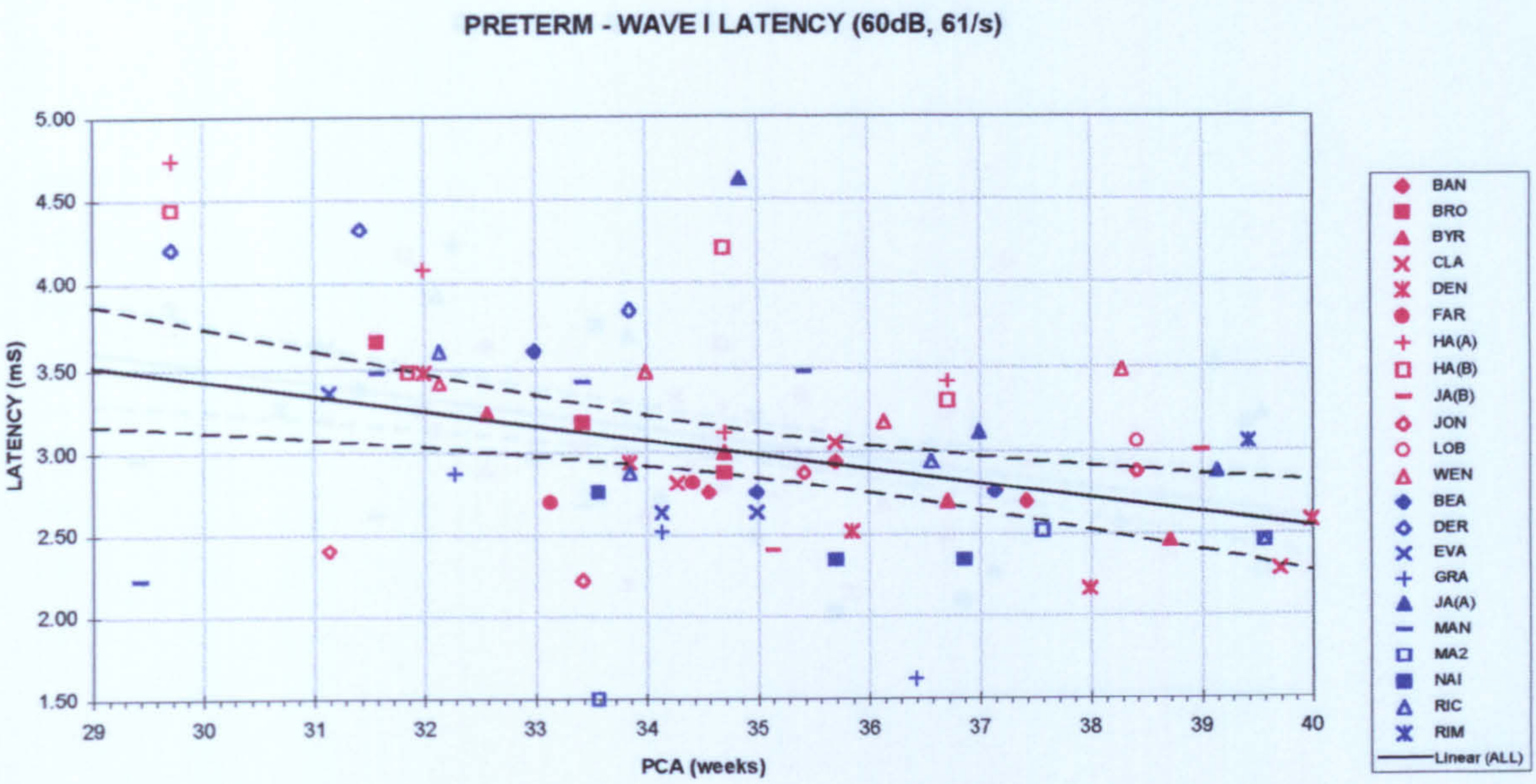


r^2=0.13 n=71 P<0.05

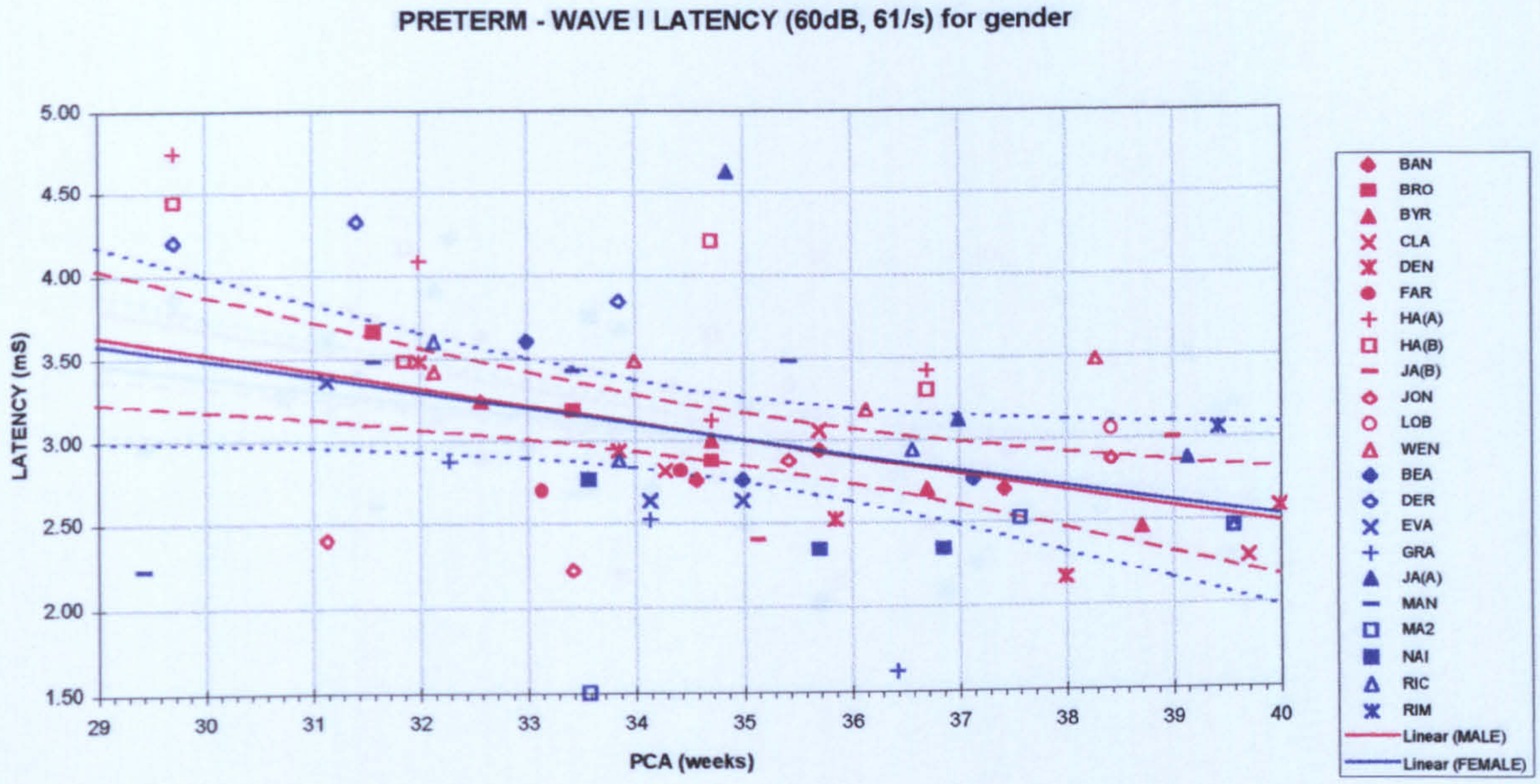


Female - r^2=0.14 n=30 P<0.05 Male - r^2=0.15 n=41 P<0.02

Figure D3 a/b

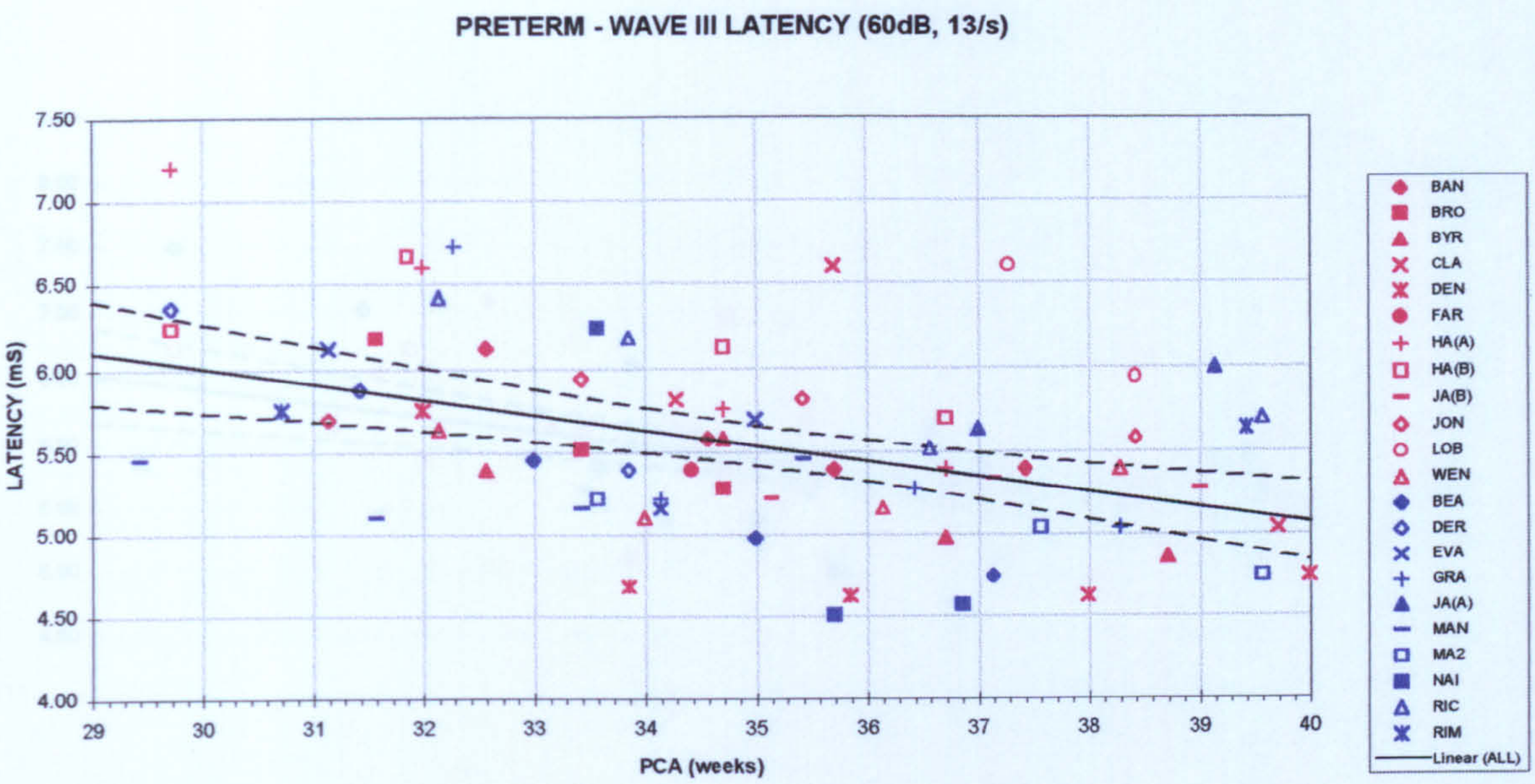


r^2=0.20 n=70 P<0.001

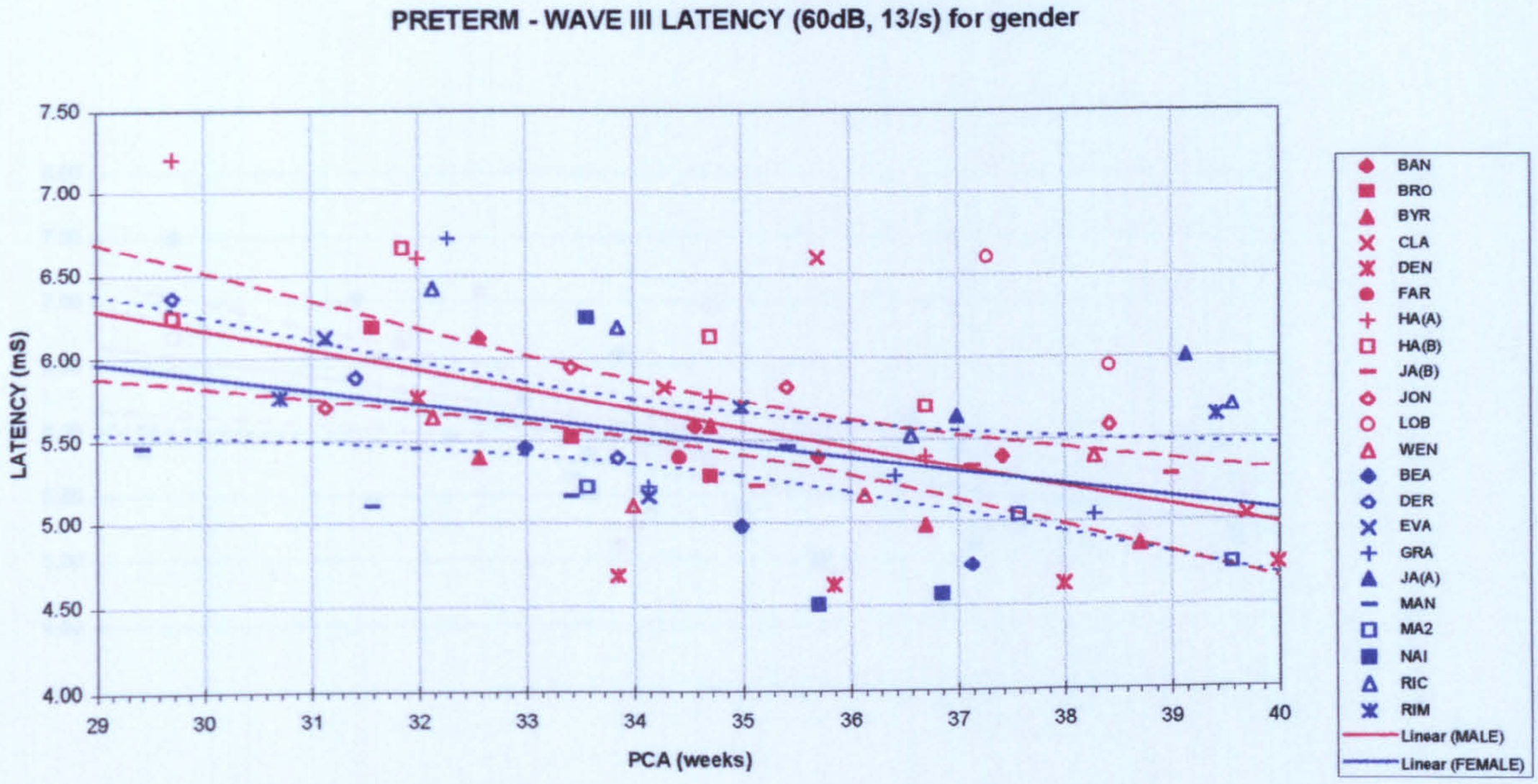


Female - r^2=0.13 n=30 P<0.05 Male - r^2=0.03 n=40 P<0.001

Figure D4 a/b

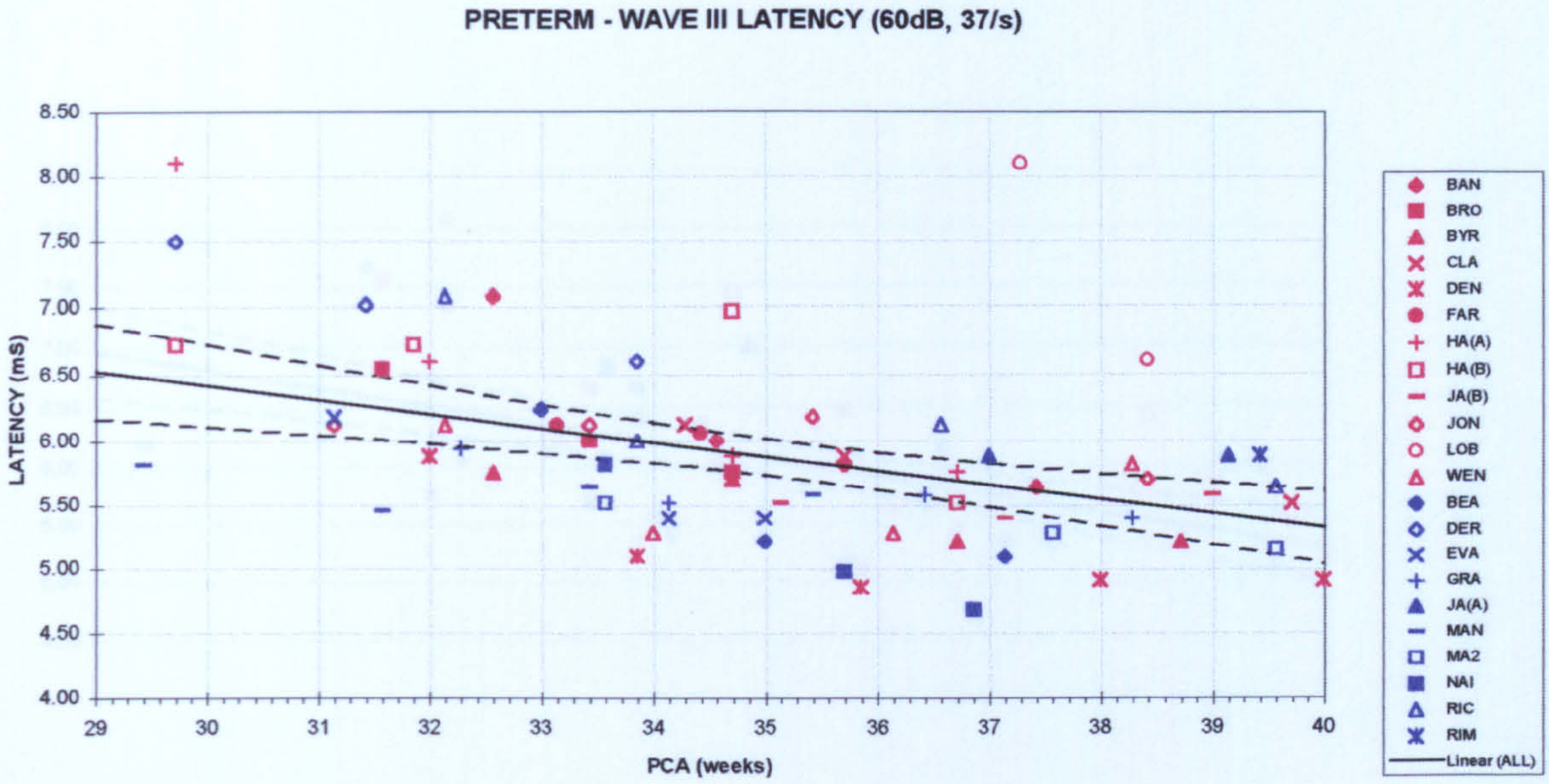


r^2=0.23 n=72 P<0.001

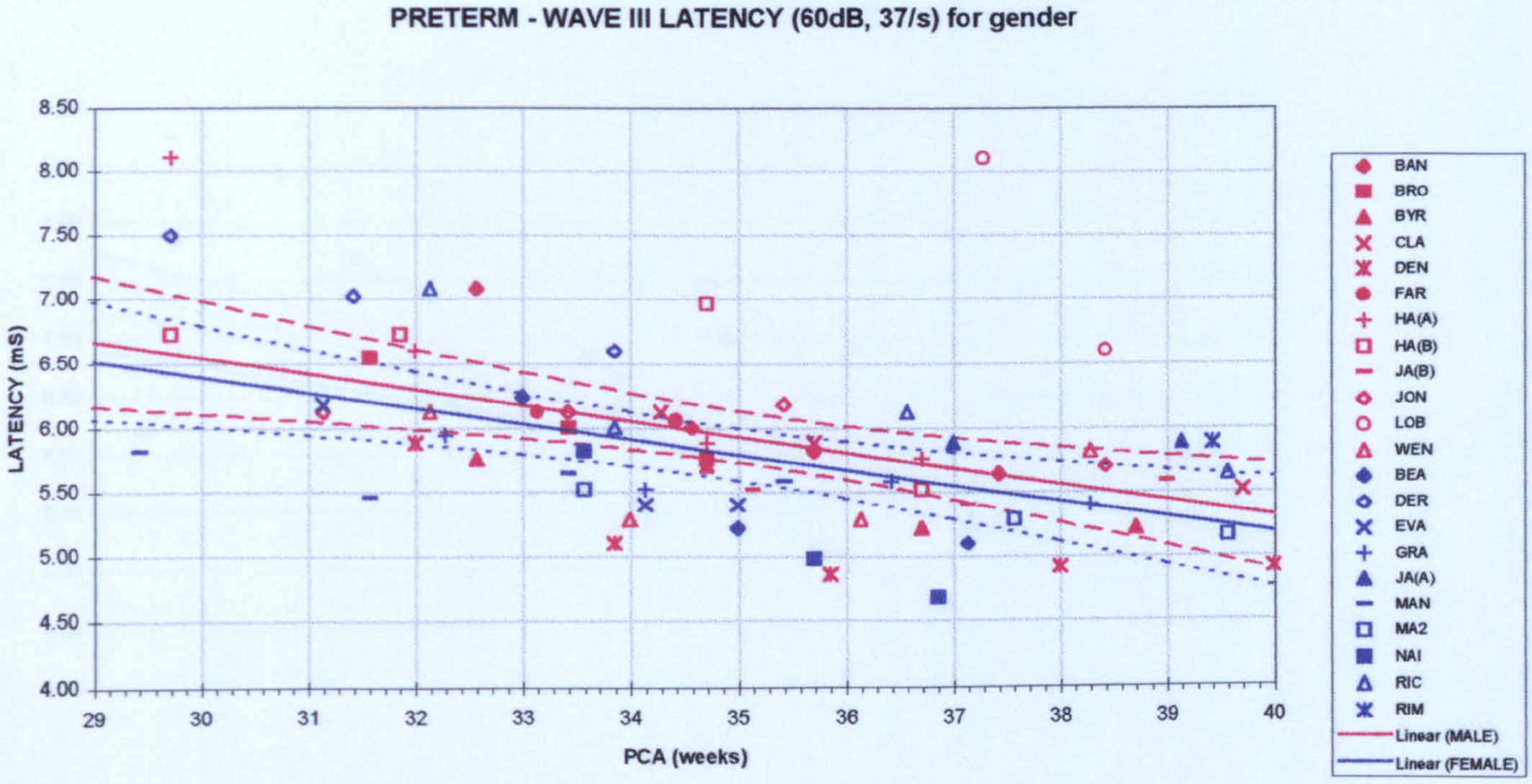


Female - r^2=0.18 n=31 P<0.025 Male - r^2=0.30 n=41 P<0.001

Figure D5 a/b

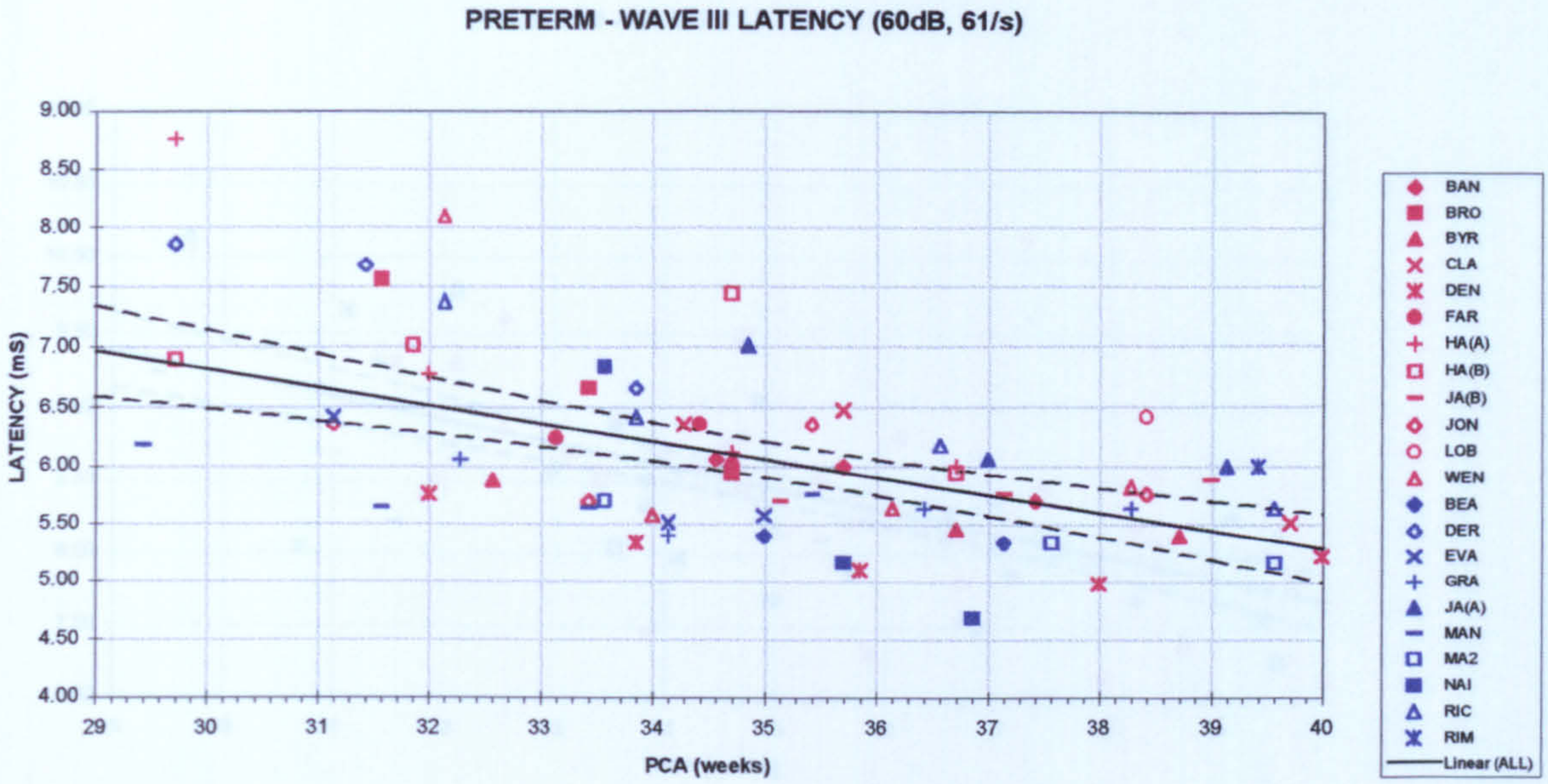


r^2=0.25 n=72 P<0.001

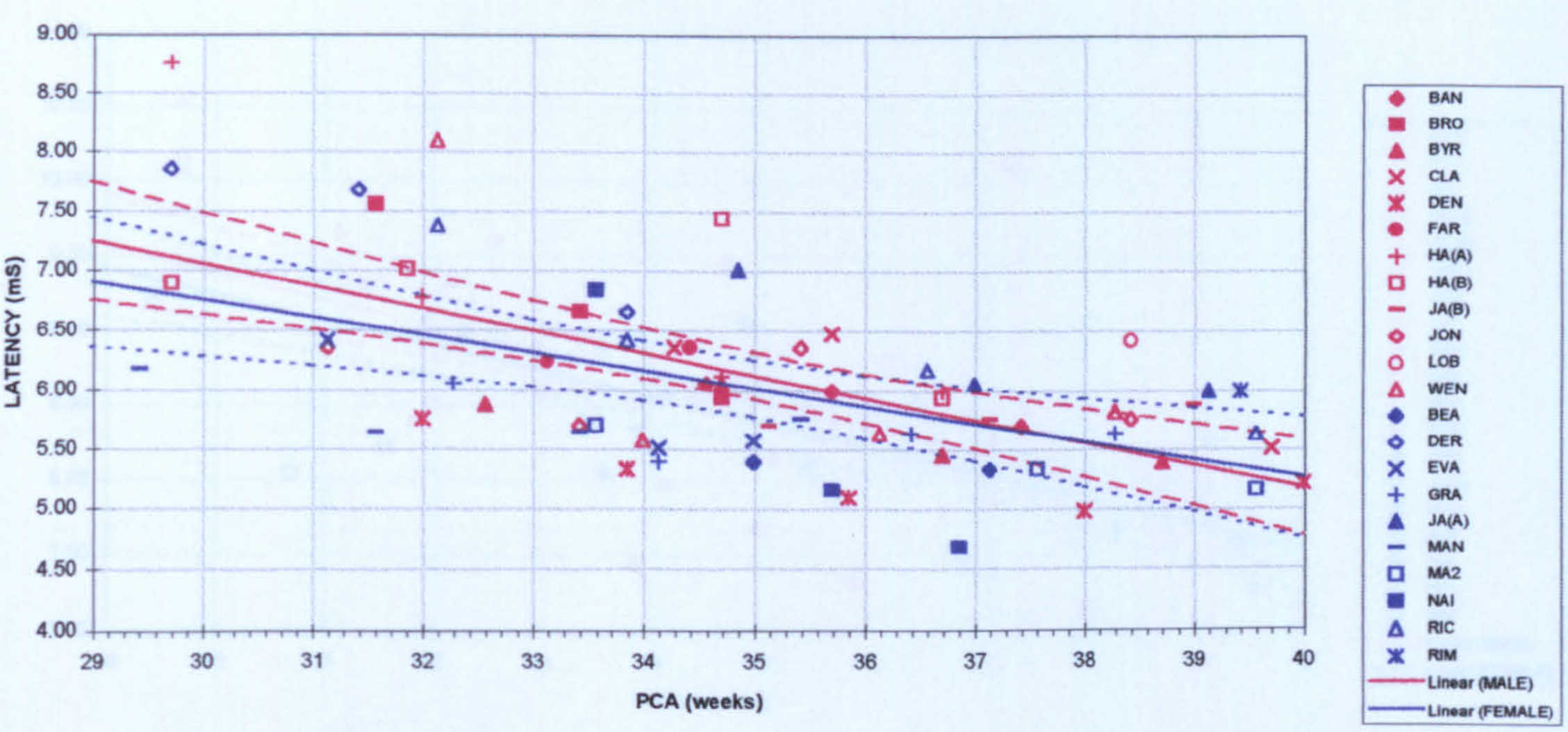


Female - r^2=0.29 n=30 P<0.005 Male - r^2=0.24 n=42 P<0.001

Figure D6 a/b

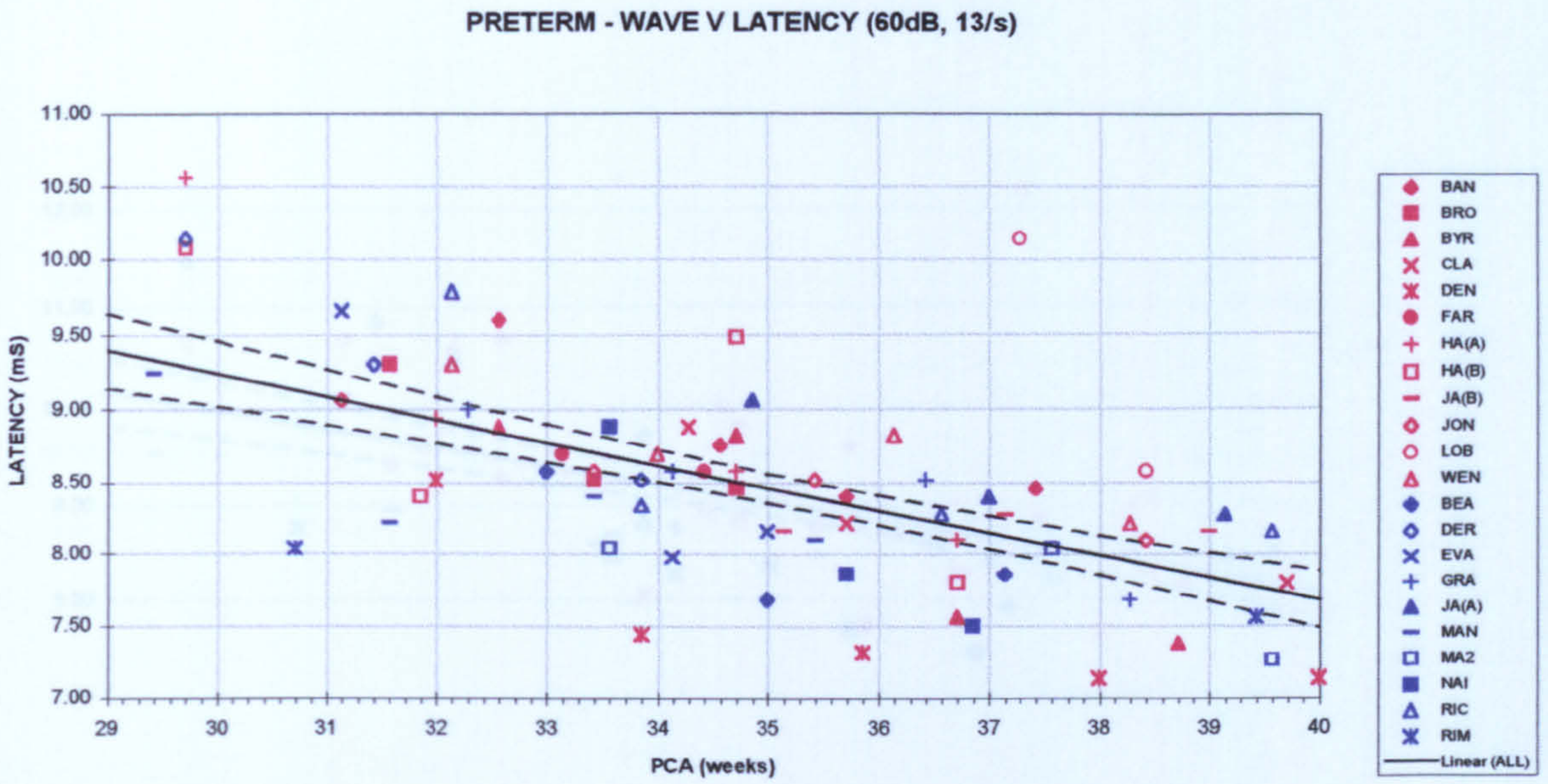


r^2=0.36 n=70 P<0.001

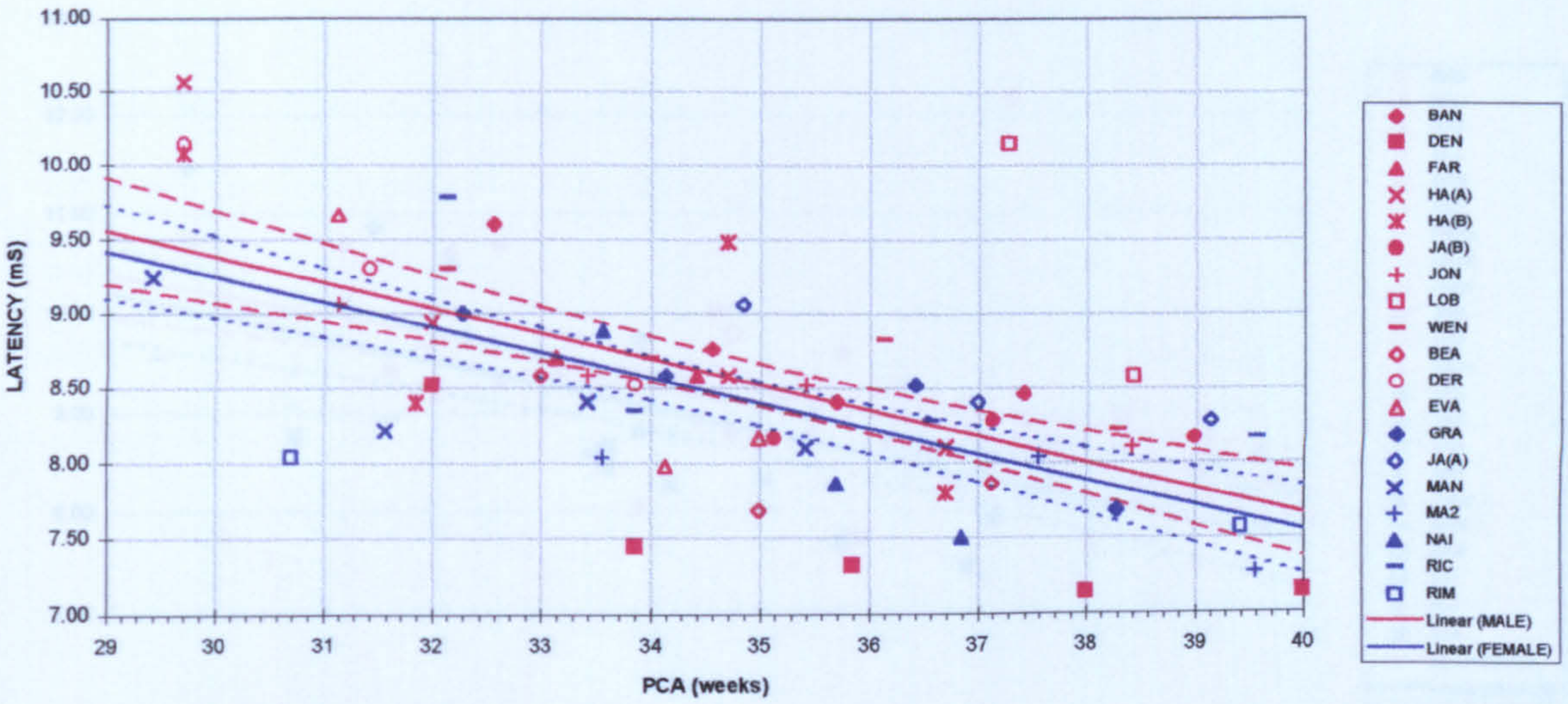


Female - r^2=0.31 n=30 P<0.005 Male - r^2=0.41 n=40 P<0.001

Figure D7 a/b

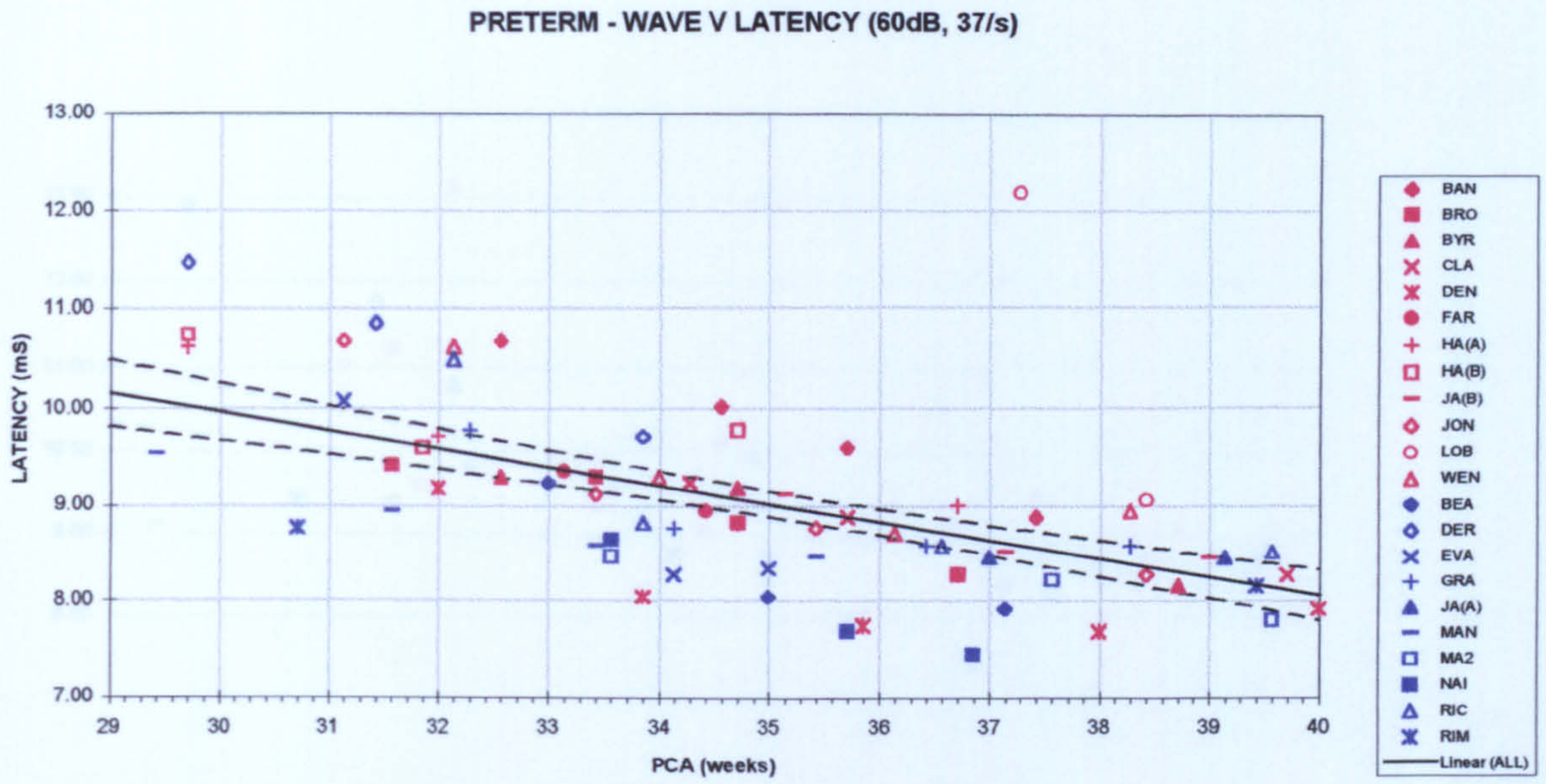


r^2=0.44 n=74 P<0.001

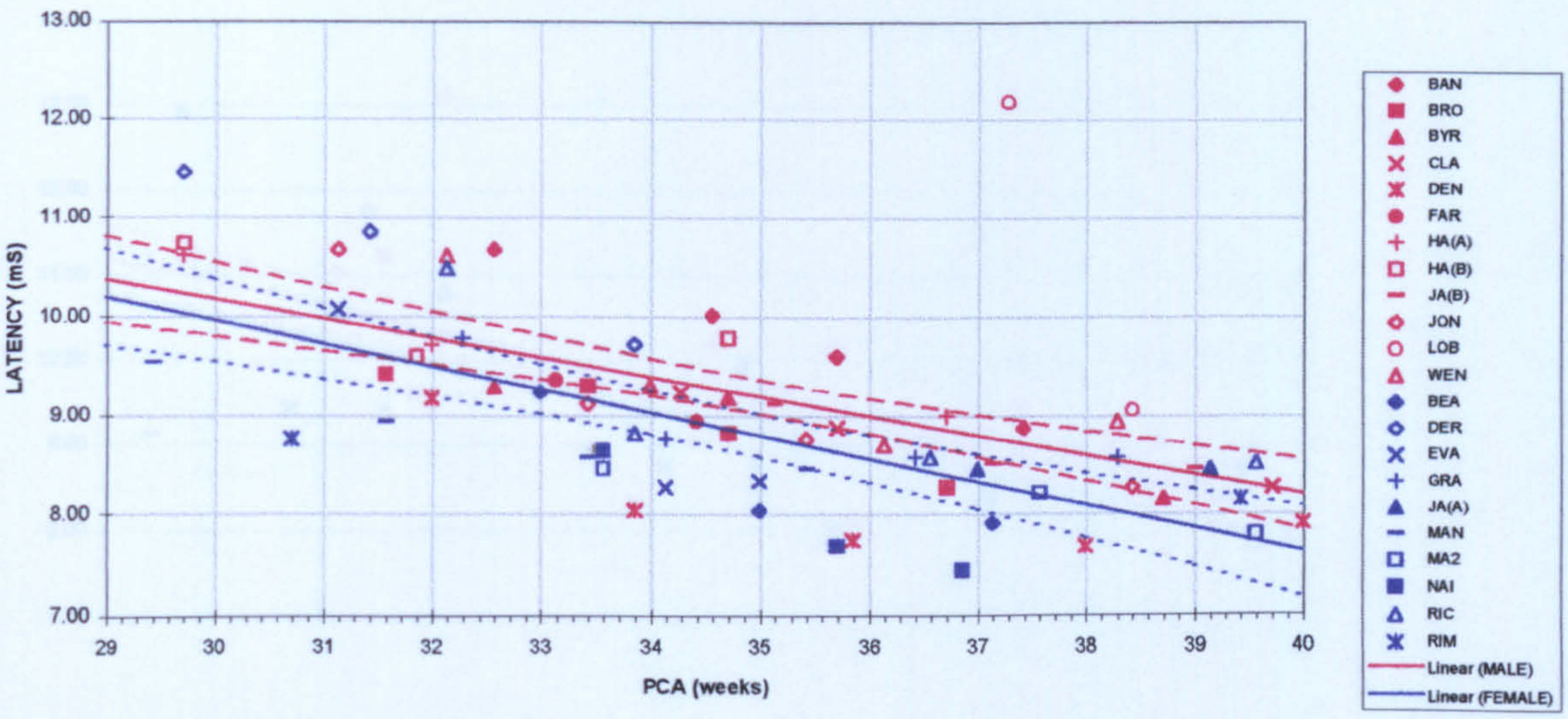


Female - r^2=0.50 n=32 P<0.001 Male - r^2=0.42 n=42 P<0.001

Figure D8 a/b

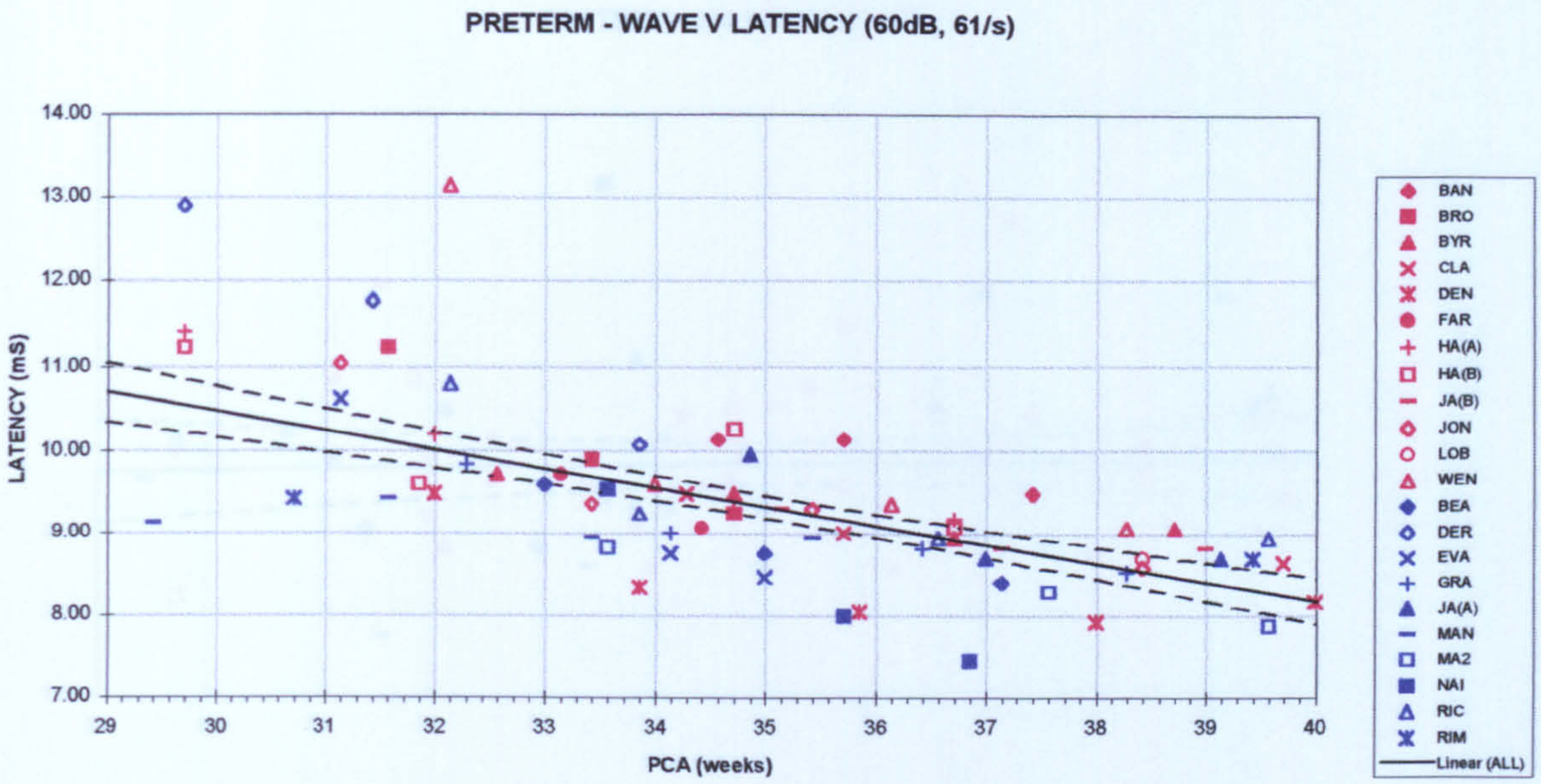


r^2=0.38 n=73 P<0.001

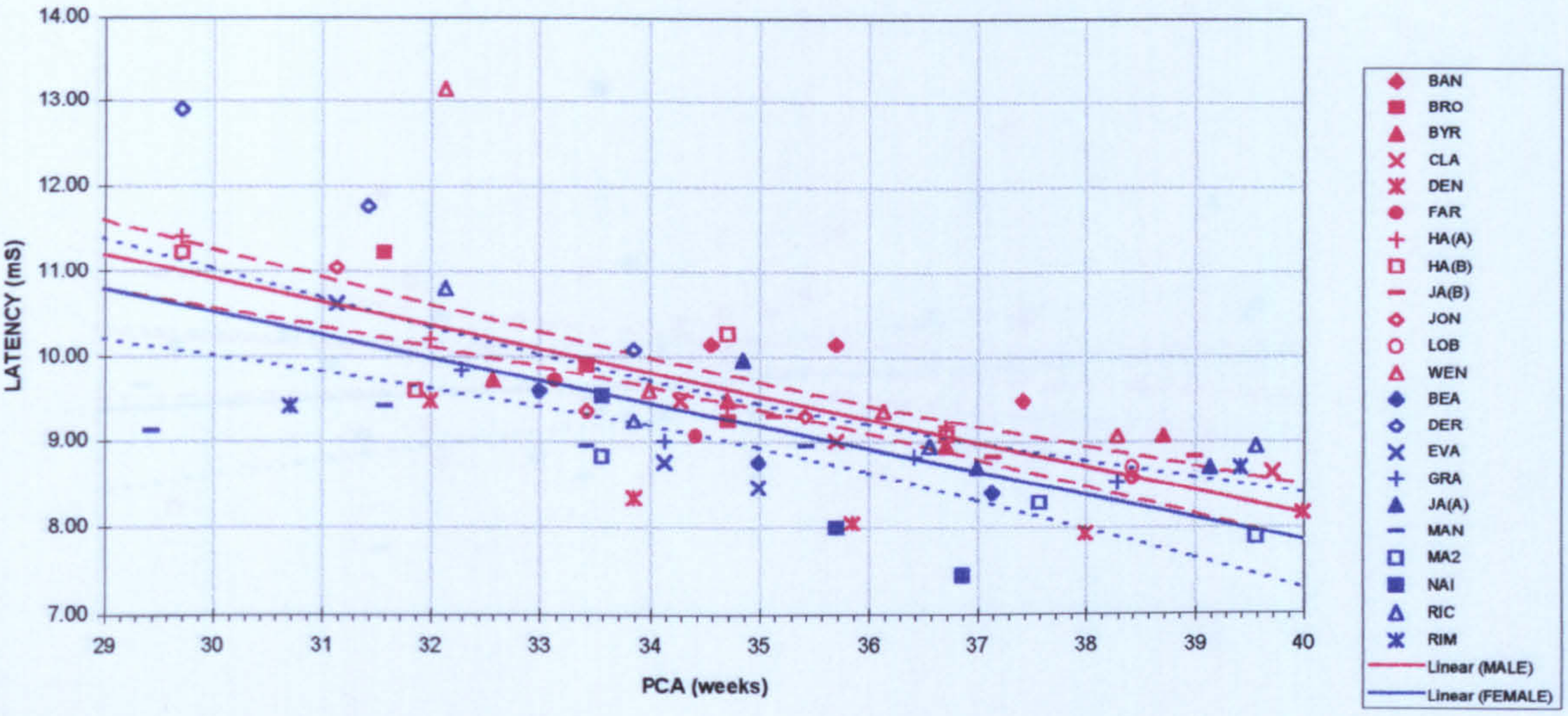


Female - r^2=0.50 n=31 P<0.001 Male - r^2=0.34 n=42 P<0.001

Figure D9 a/b

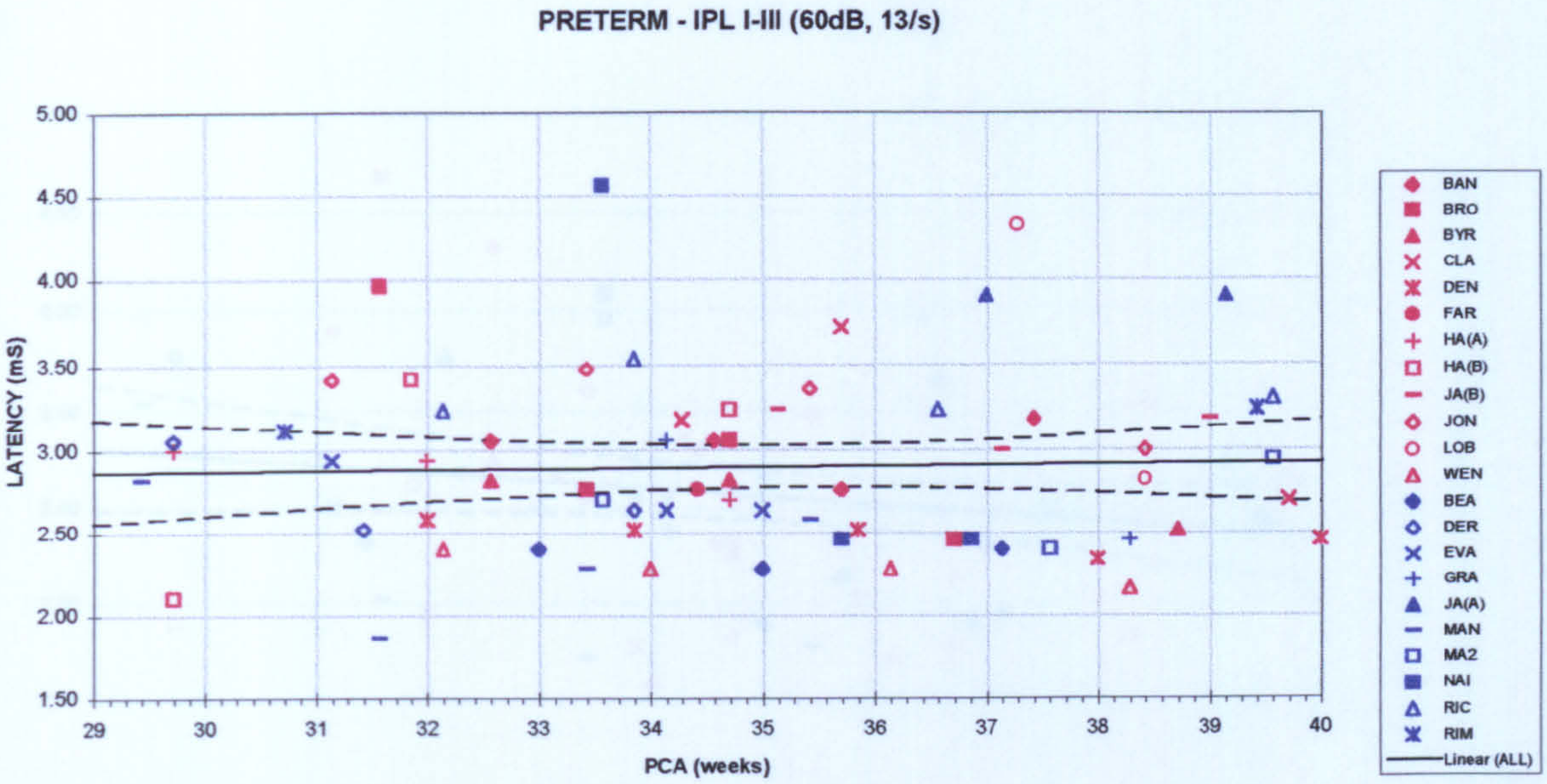


r^2=0.47 n=72 P<0.001

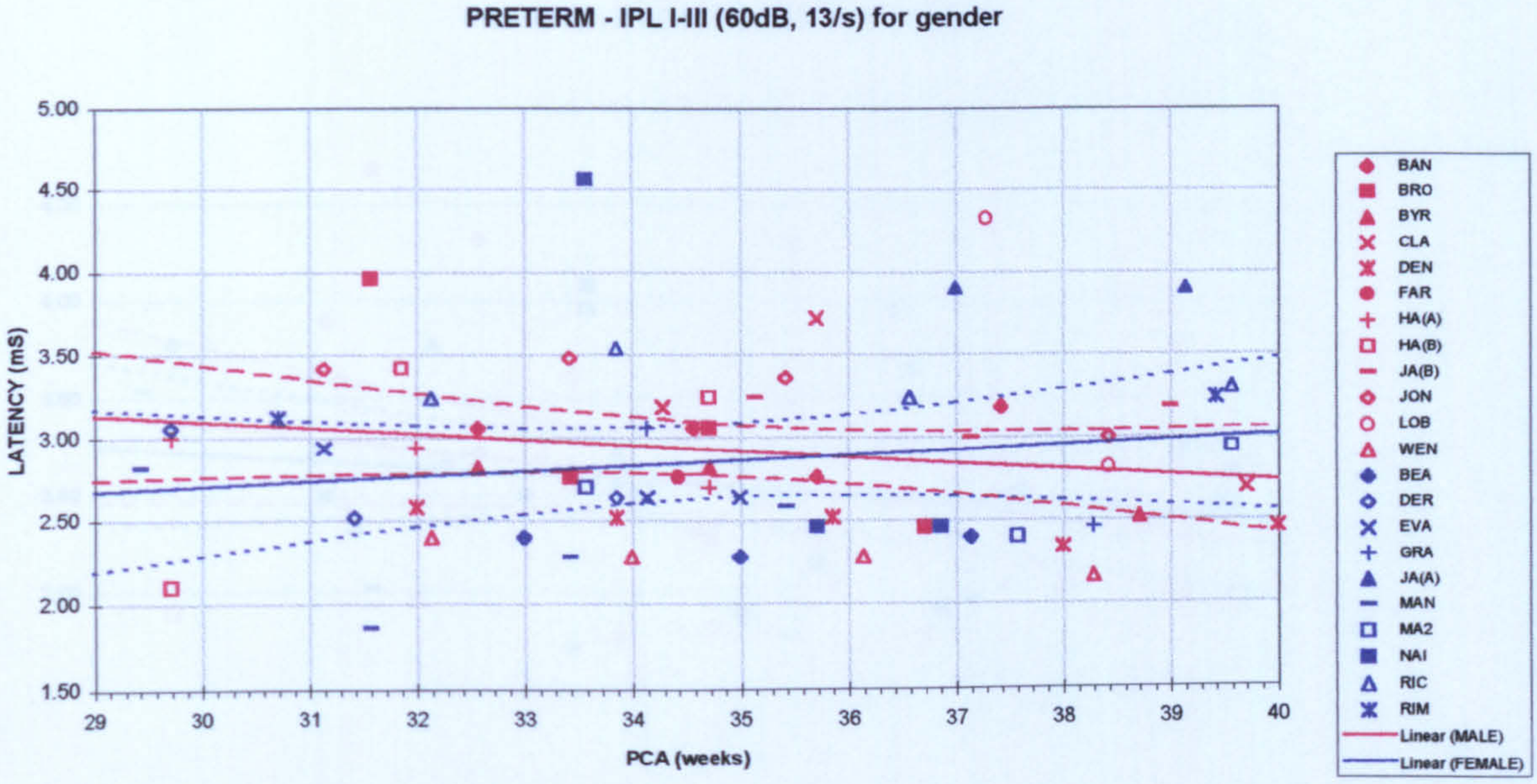


Female - r^2=0.46 n=32 P<0.001 Male - r^2=0.52 n=40 P<0.001

Figure D10 a/b

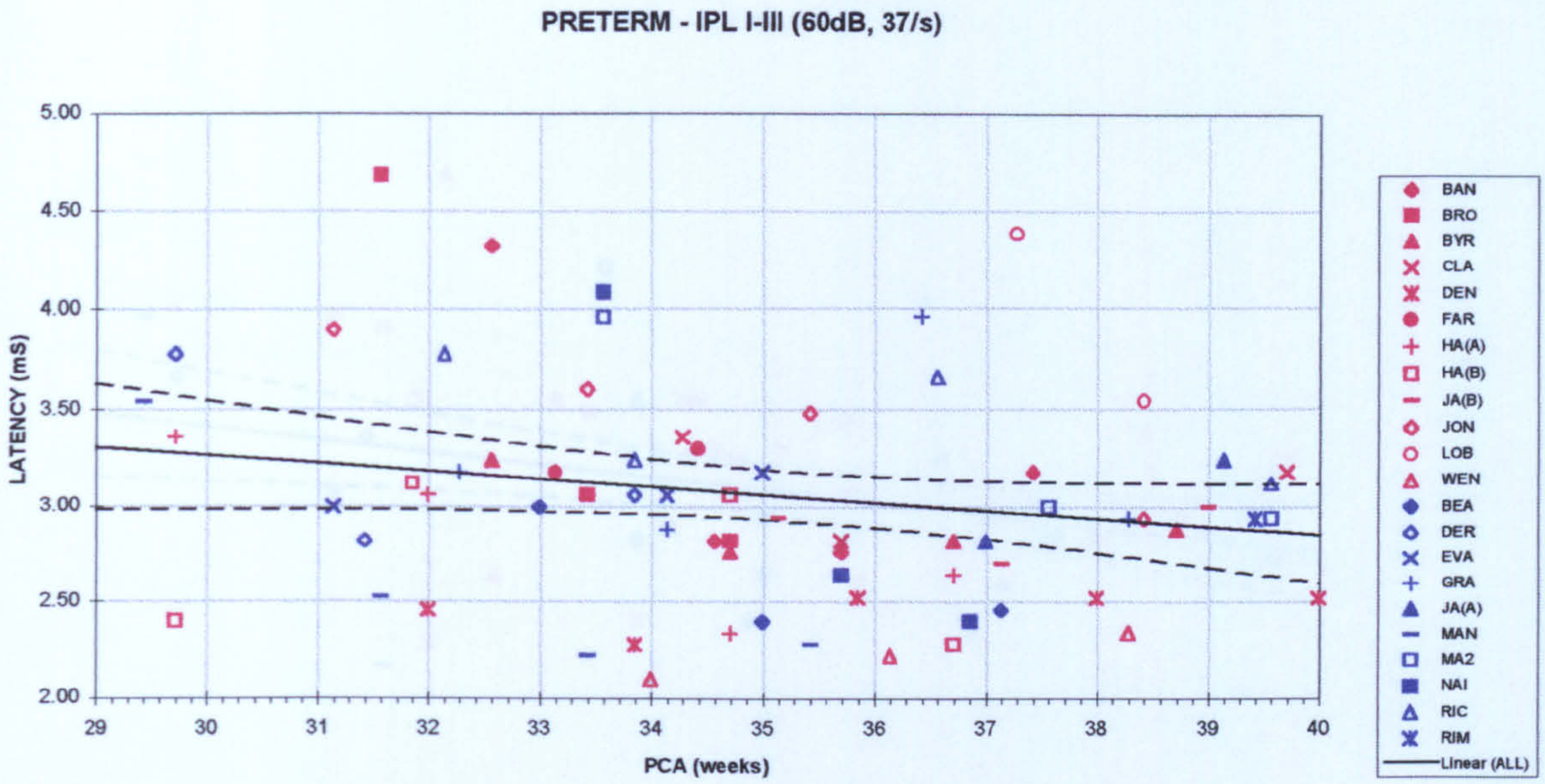


r^2=0.00 n=70 P>0.05

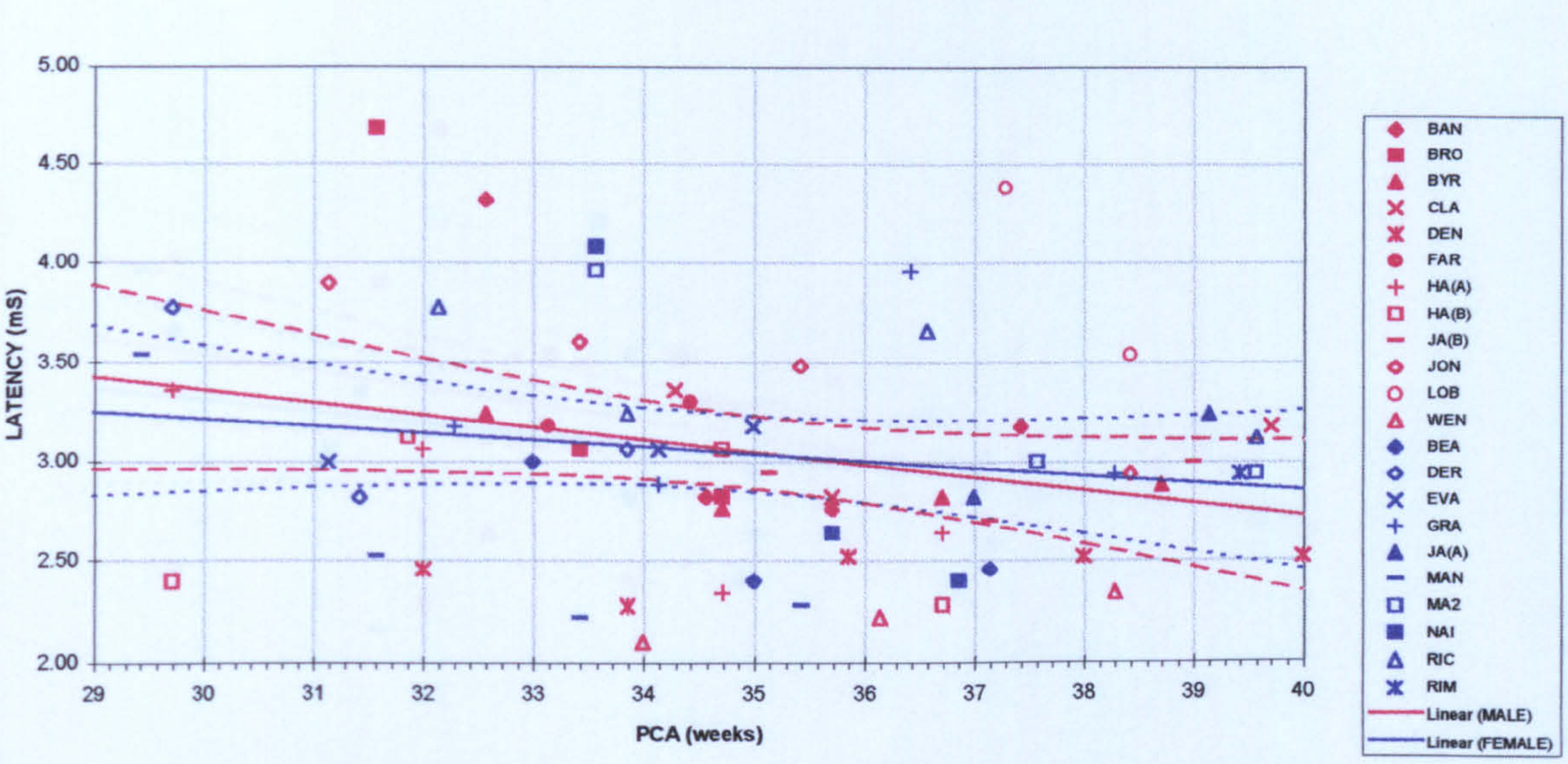


Female - r^2=0.02 n=29 P>0.05 Male - r^2=0.02 n=41 P>0.05

Figure D11 a/b

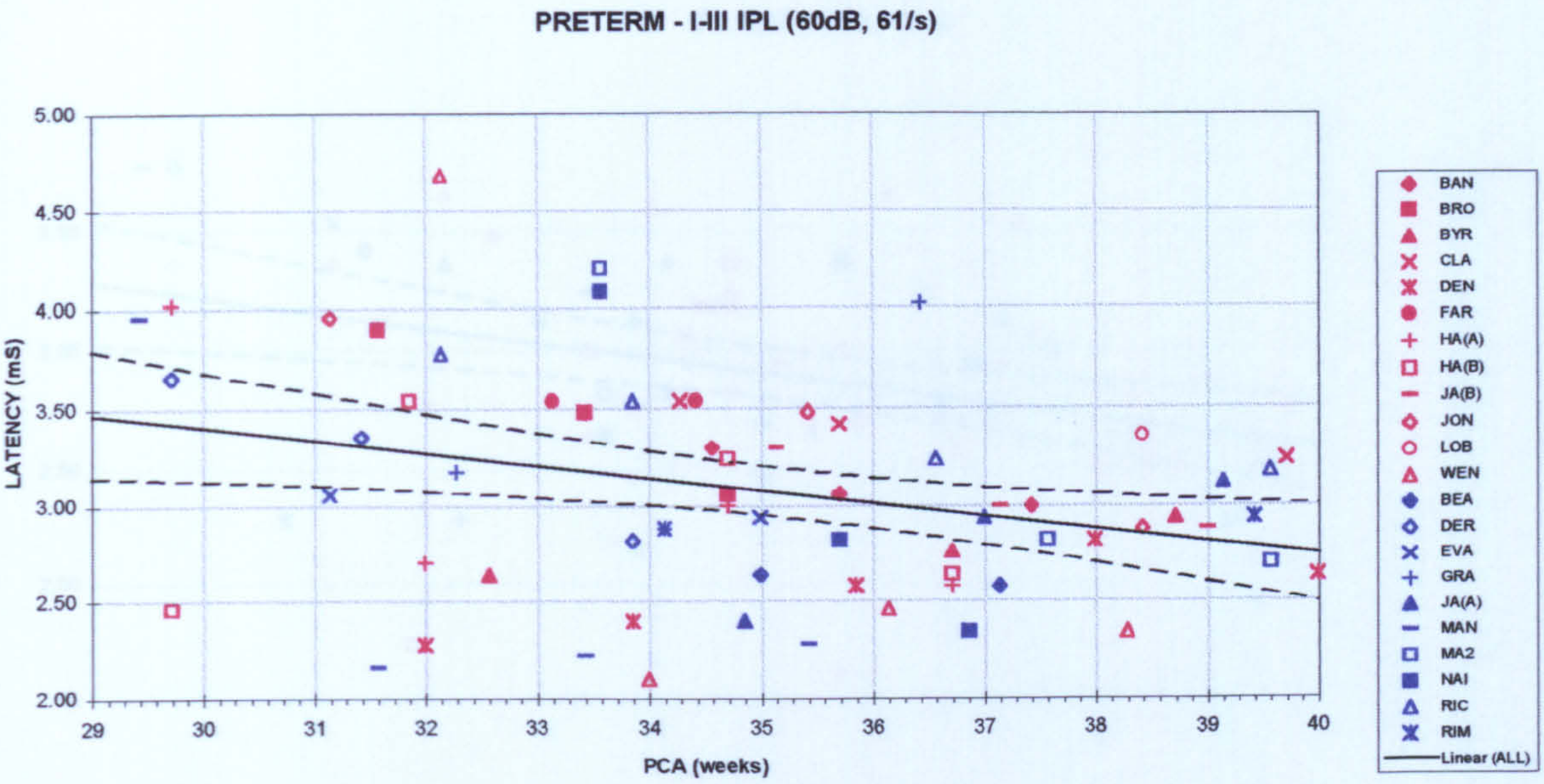


r^2=0.05 n=71 P>0.05

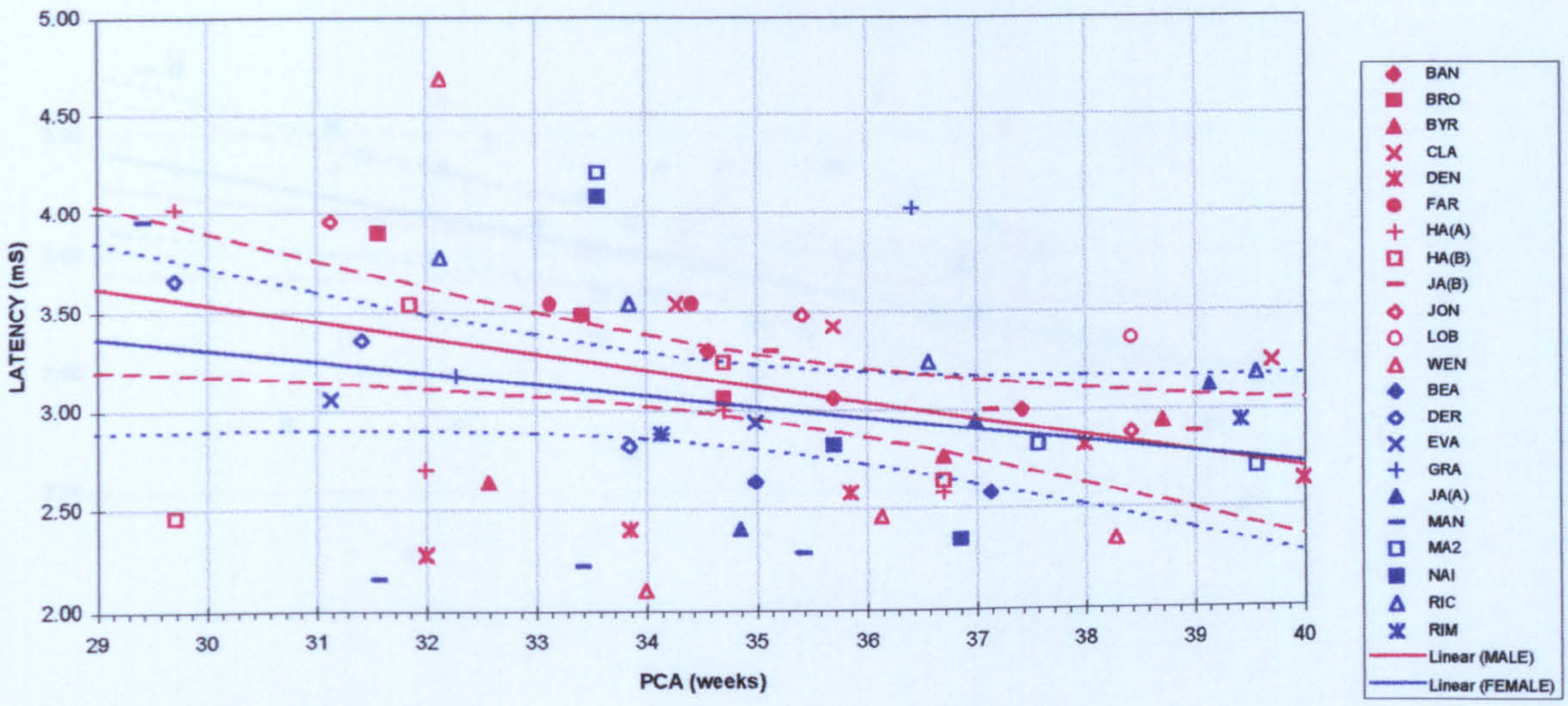


Female - r^2=0.05 n=30 P>0.05 Male - r^2=0.06 n=41 P>0.05

Figure D12 a/b

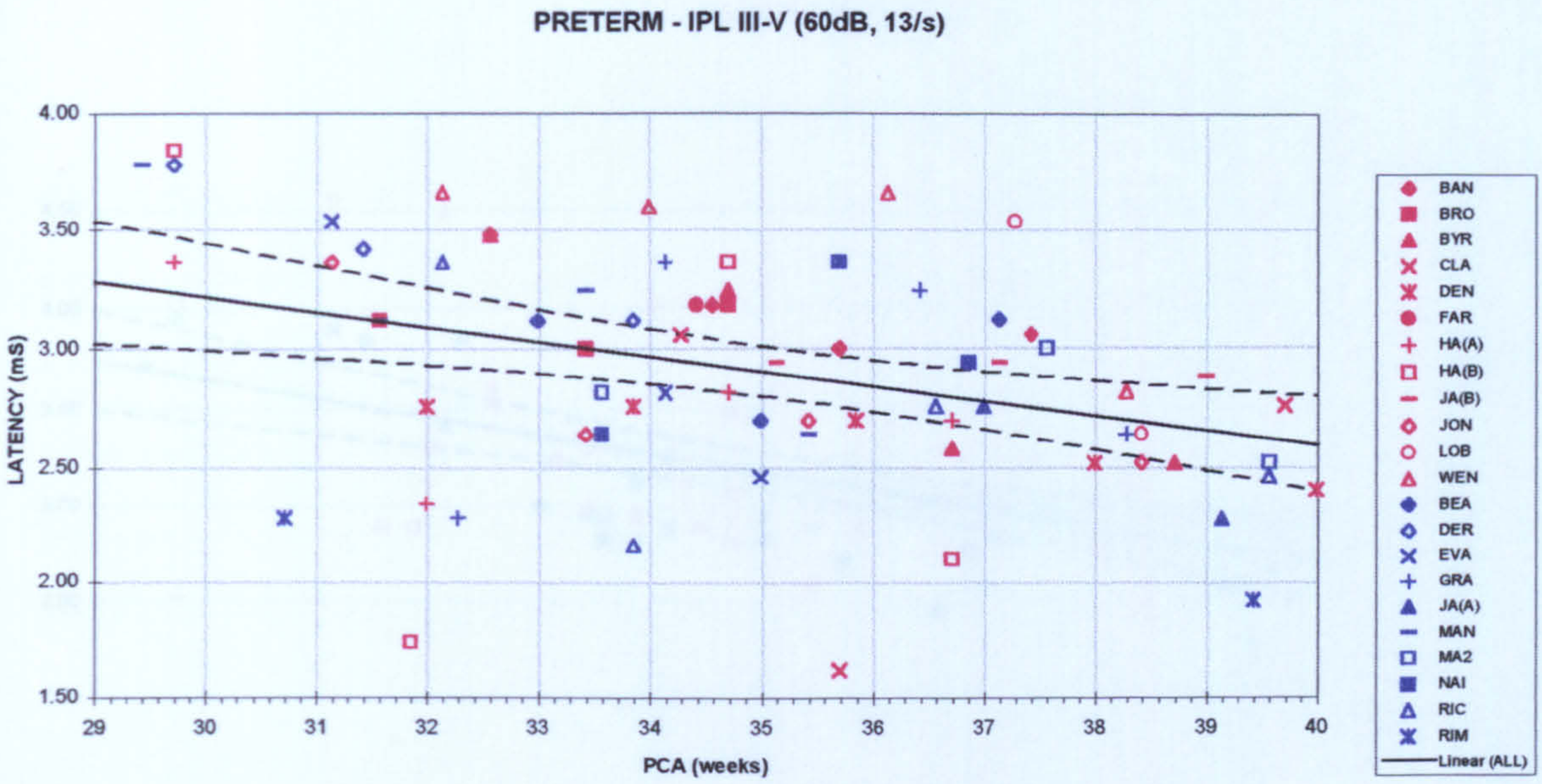


r^2=0.10 n=69 P<0.01

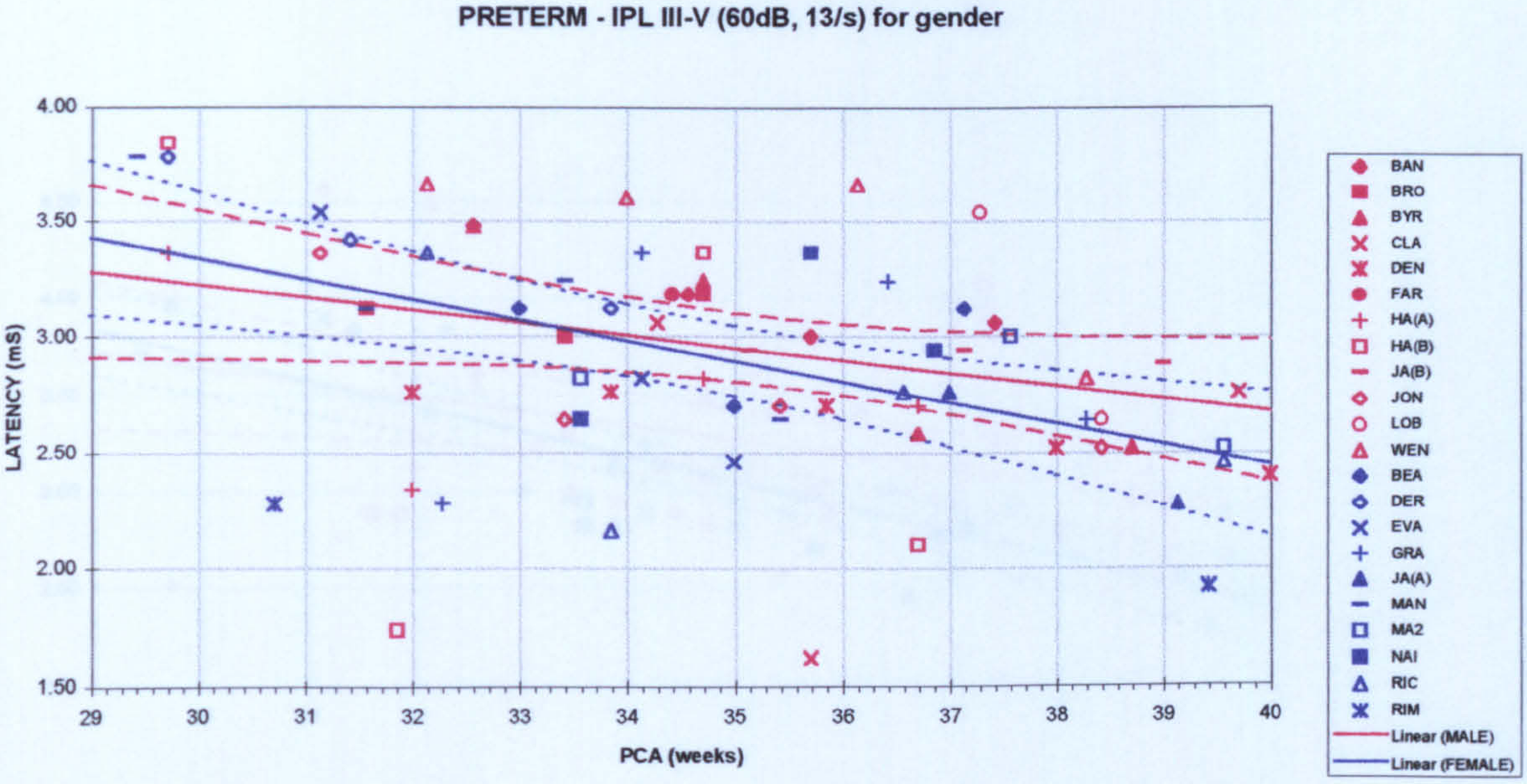


Female - r^2=0.09 n=29 P>0.05 Male - r^2=0.12 n=40 P<0.05

Figure D13 a/b

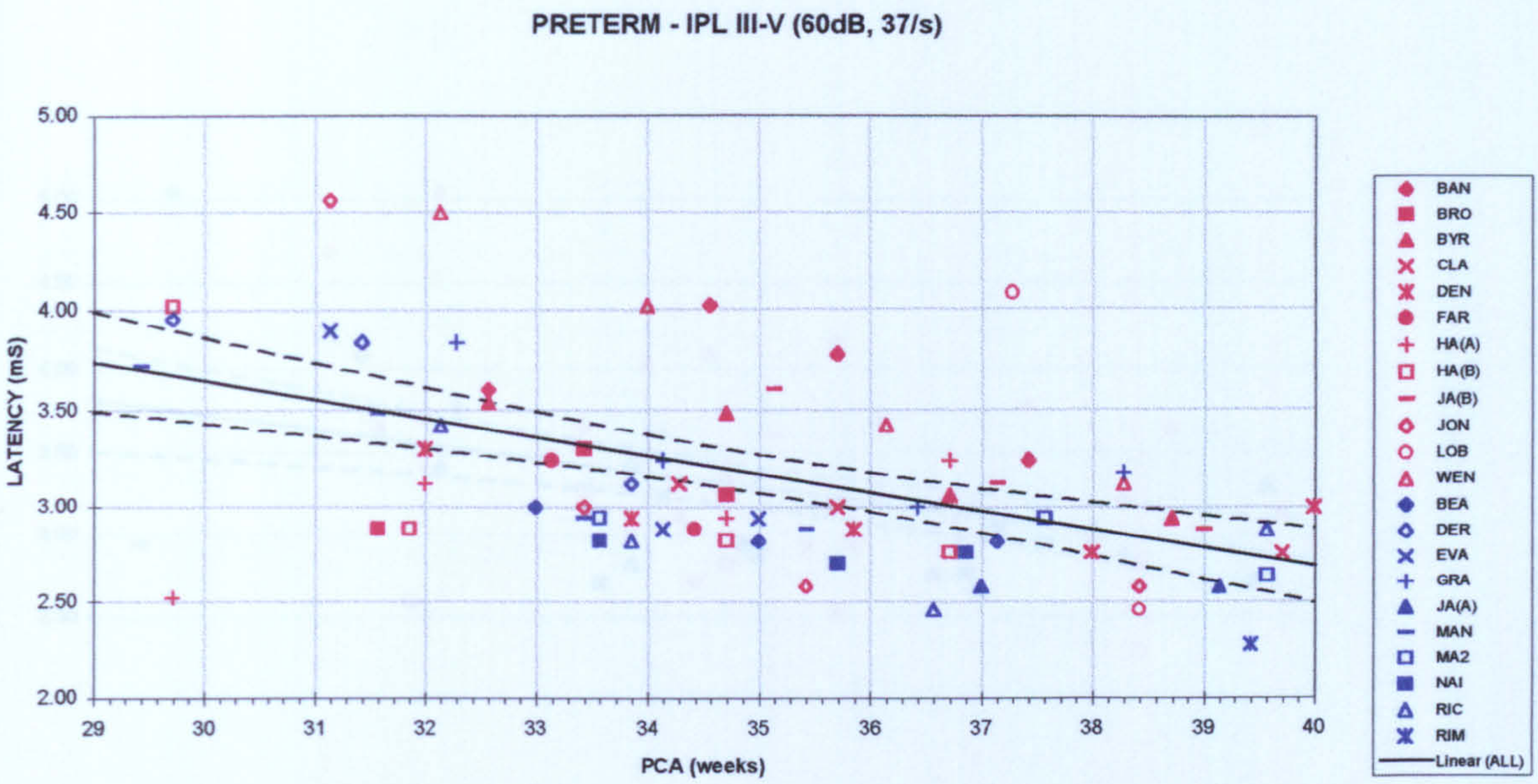


r^2=0.19 n=72 P<0.001

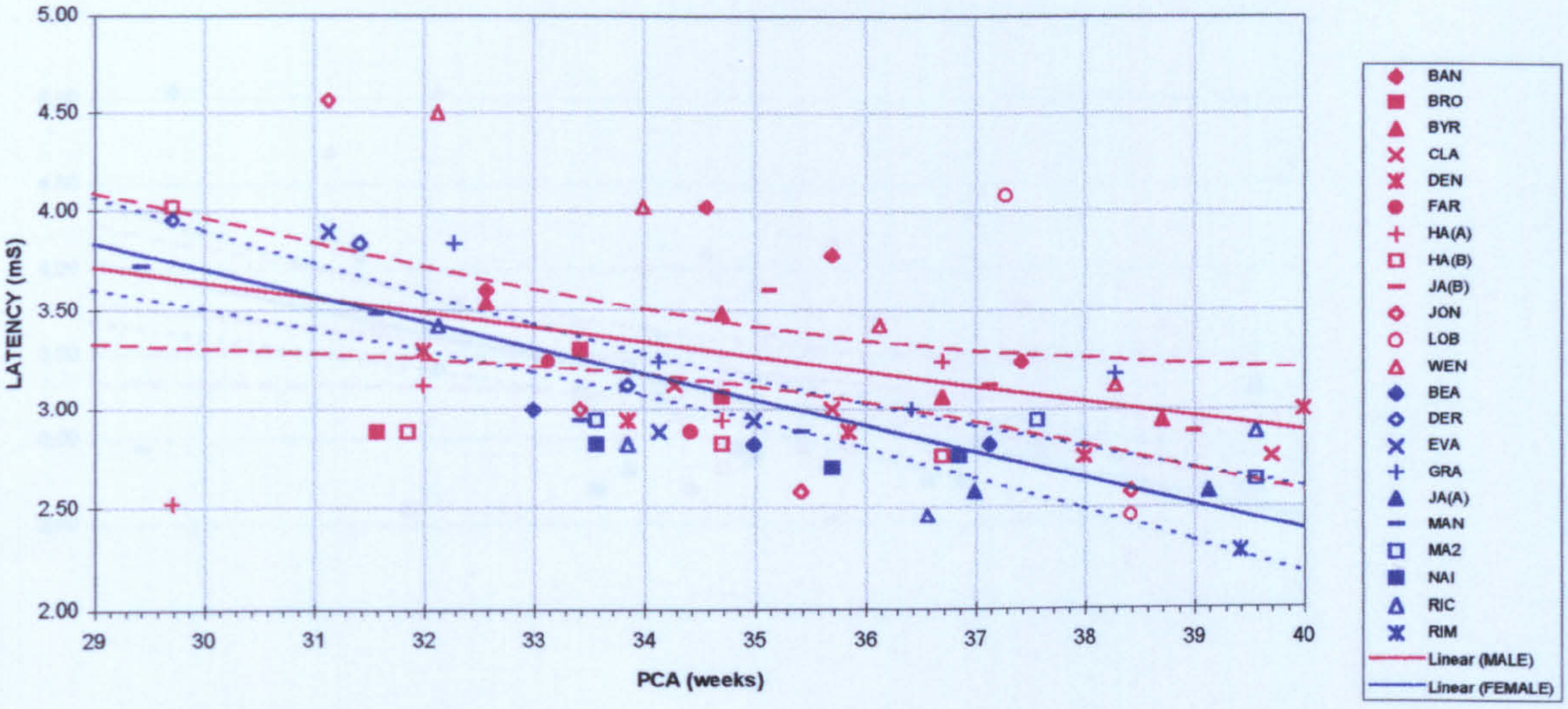


Female - r^2=0.29 n=31 P<0.005 Male - r^2=0.13 n=41 P<0.025

Figure D14 a/b

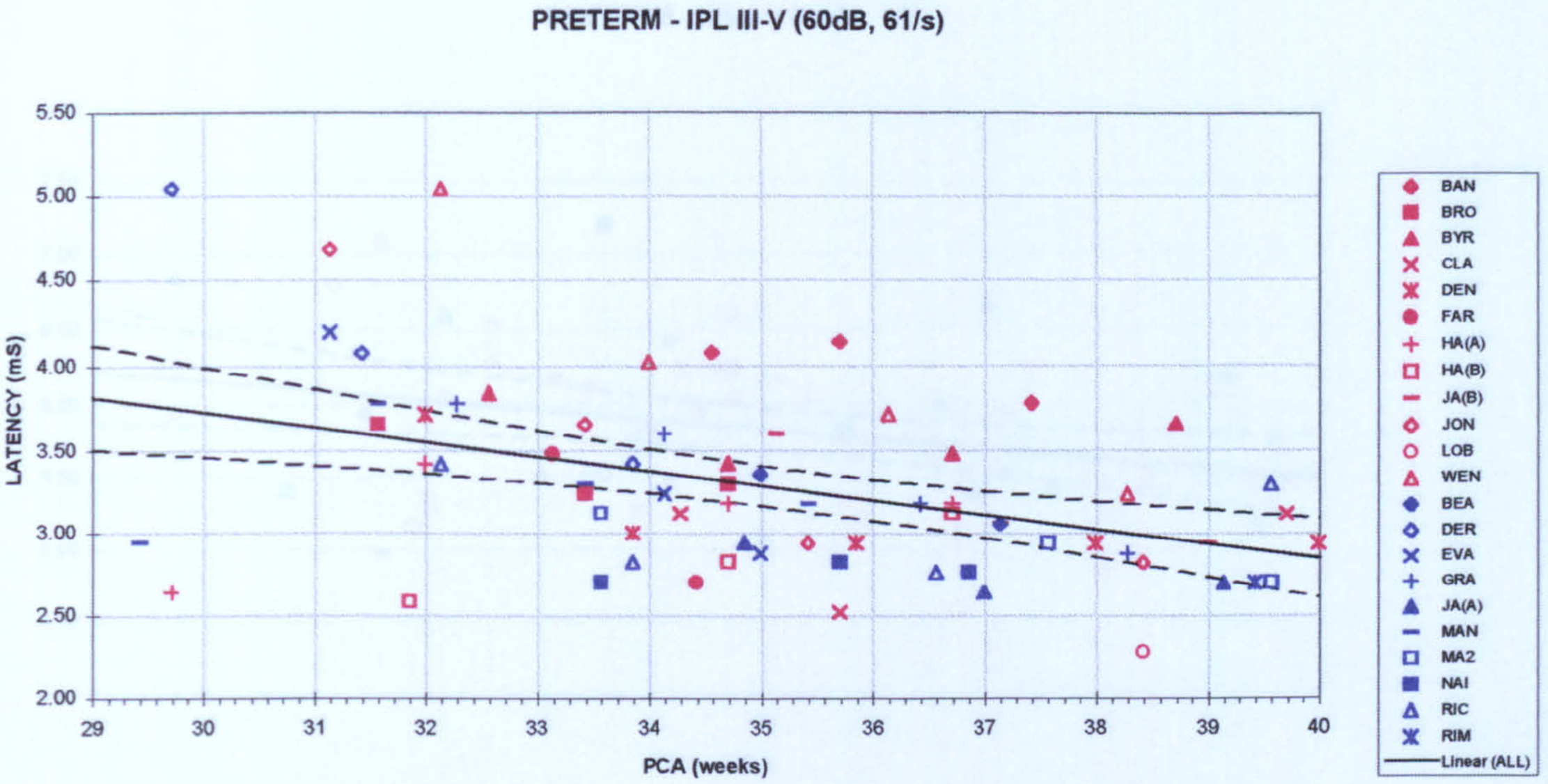


r^2=0.27 n=72 P<0.001

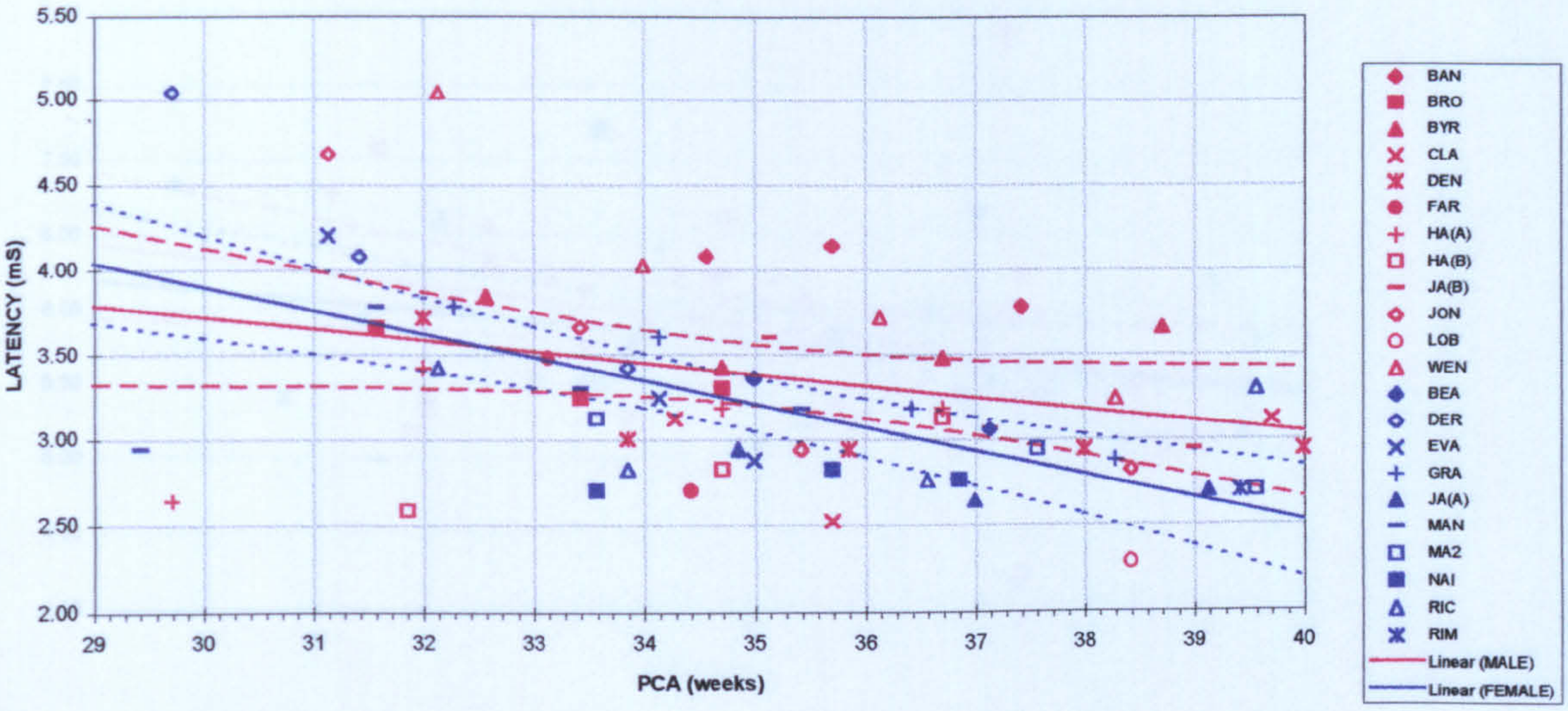


Female - r^2=0.63 n=30 P<0.001 Male - r^2=0.13 n=42 P<0.02

Figure D15 a/b

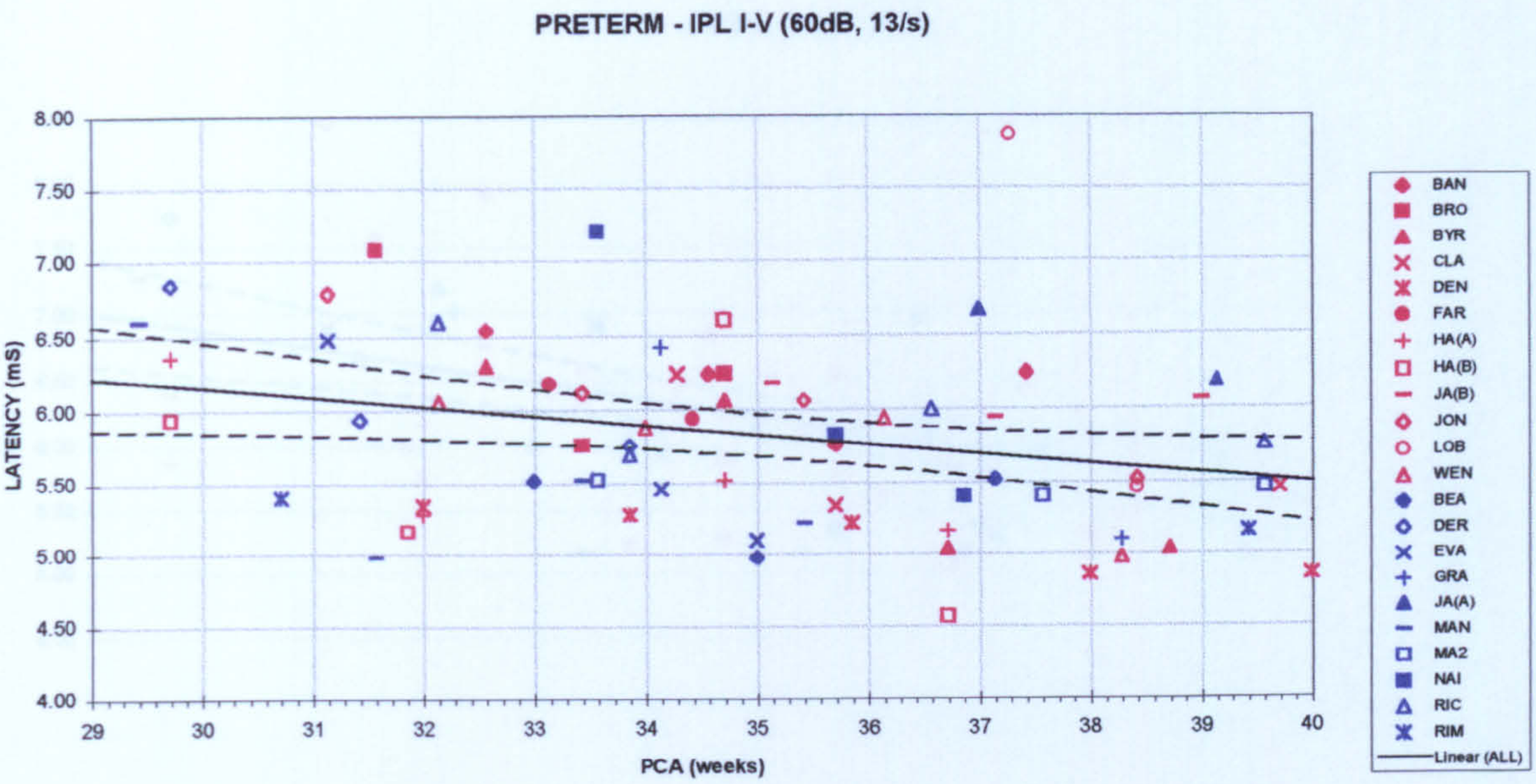


r^2=0.22 n=69 P<0.001

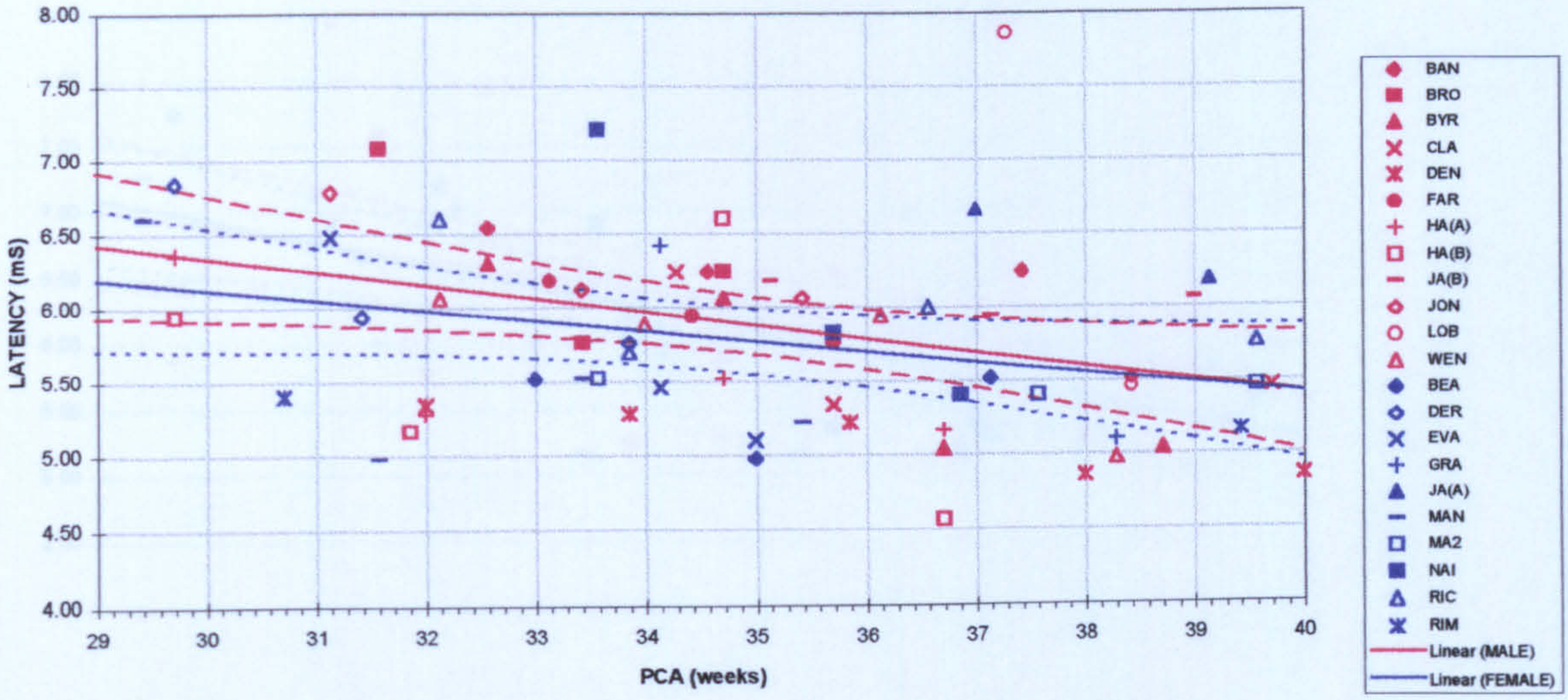


Female - r^2=0.44 n=30 P<0.001 Male - r^2=0.11 n=39 P<0.05

Figure D16 a/b

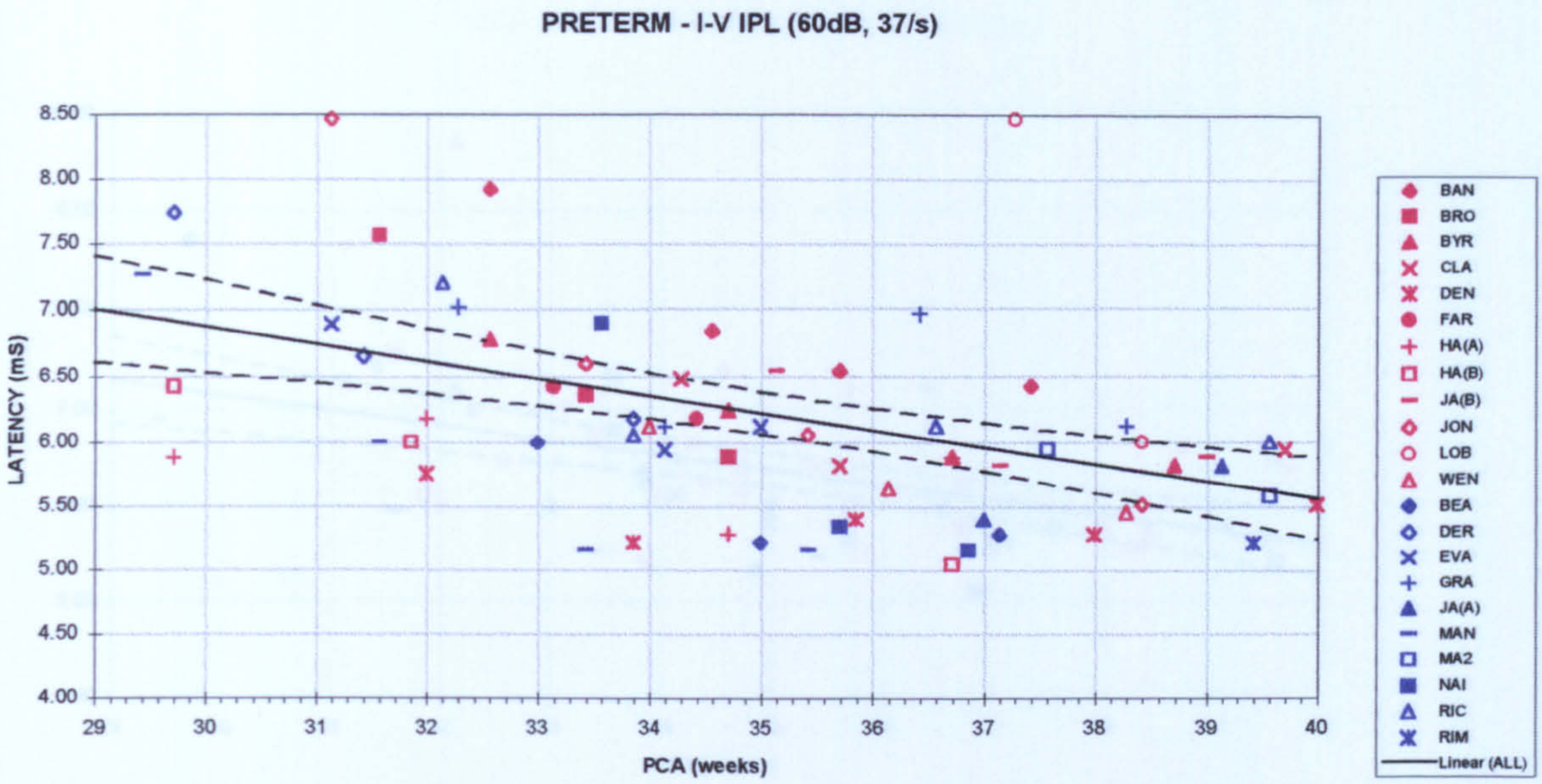


r^2=0.13 n=71 P<0.005

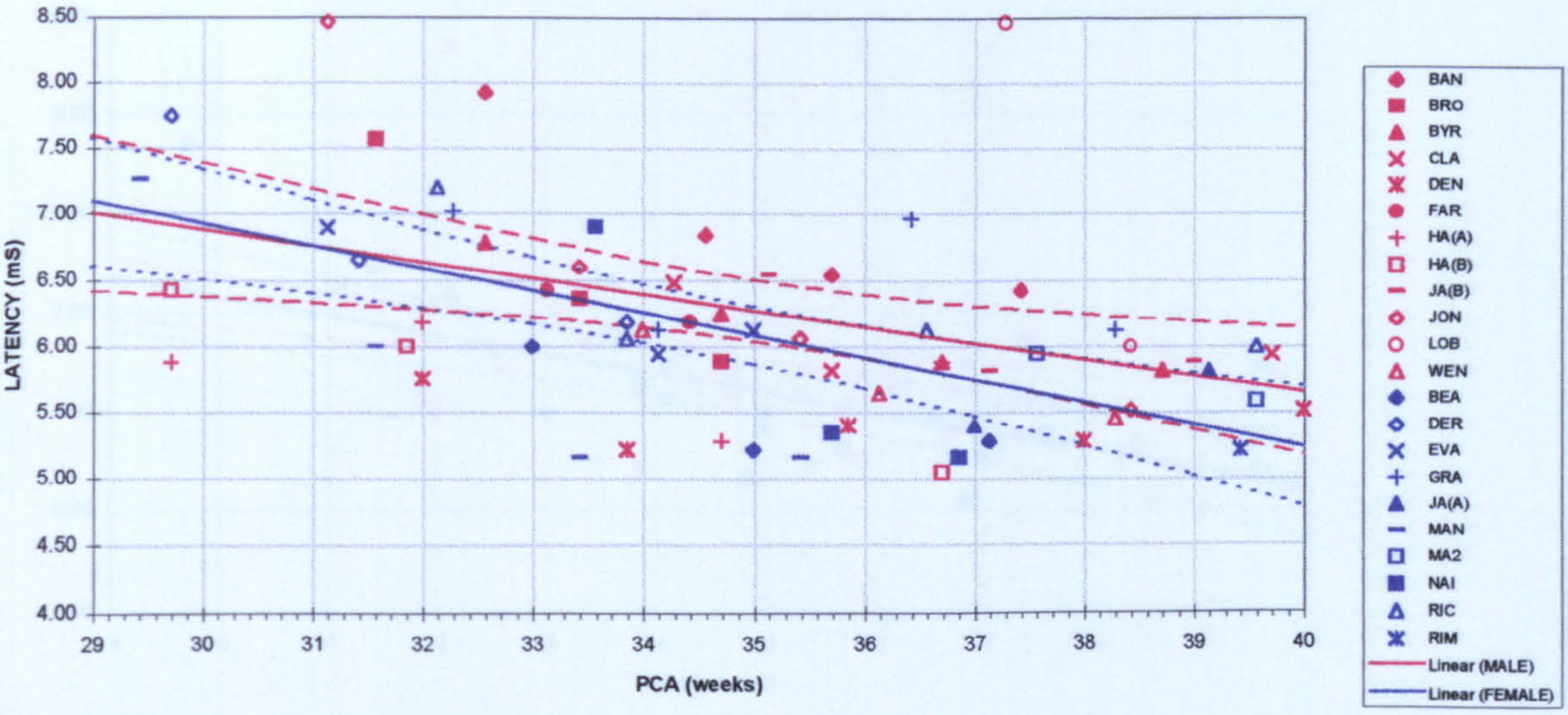


Female - r^2=0.12 n=29 P>0.05 Male - r^2=0.14 n=42 P<0.02

Figure D17 a/b

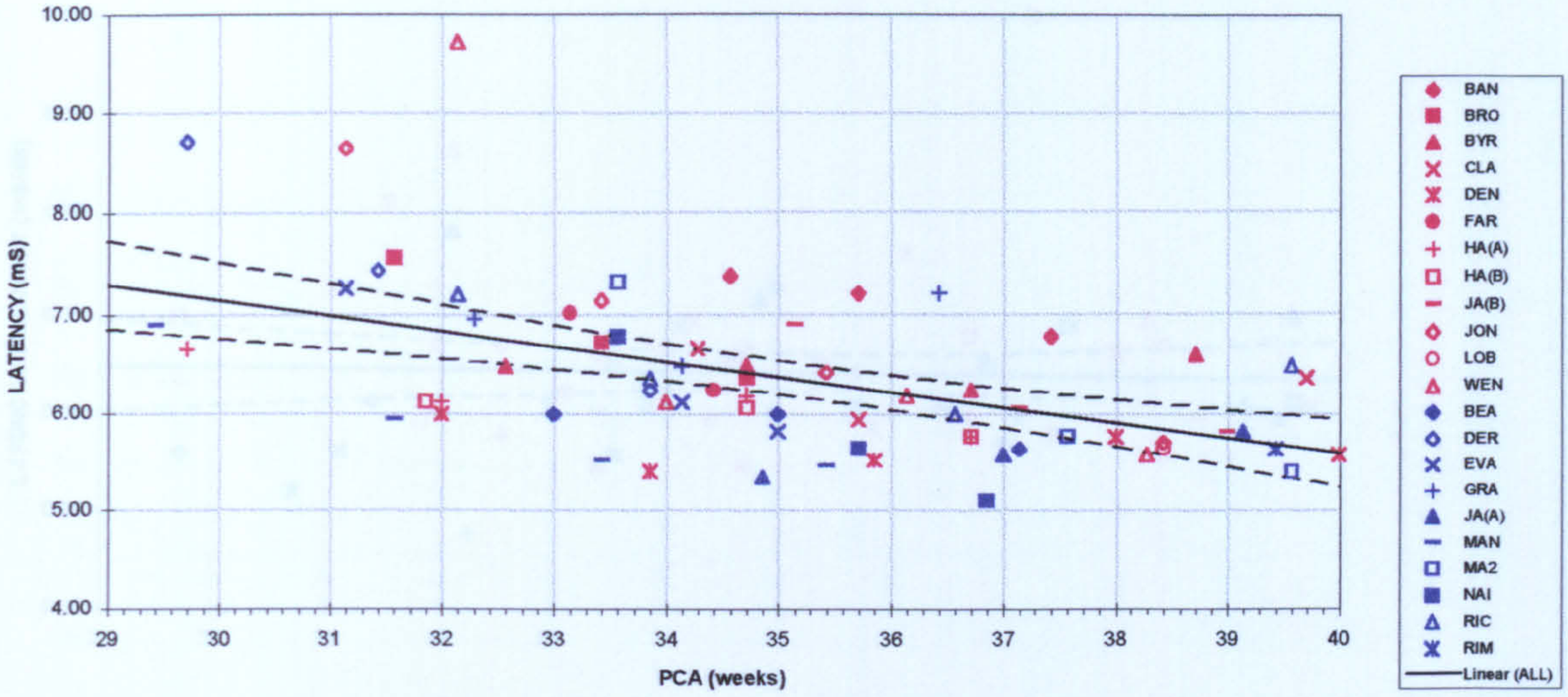


r^2=0.24 n=71 P<0.001

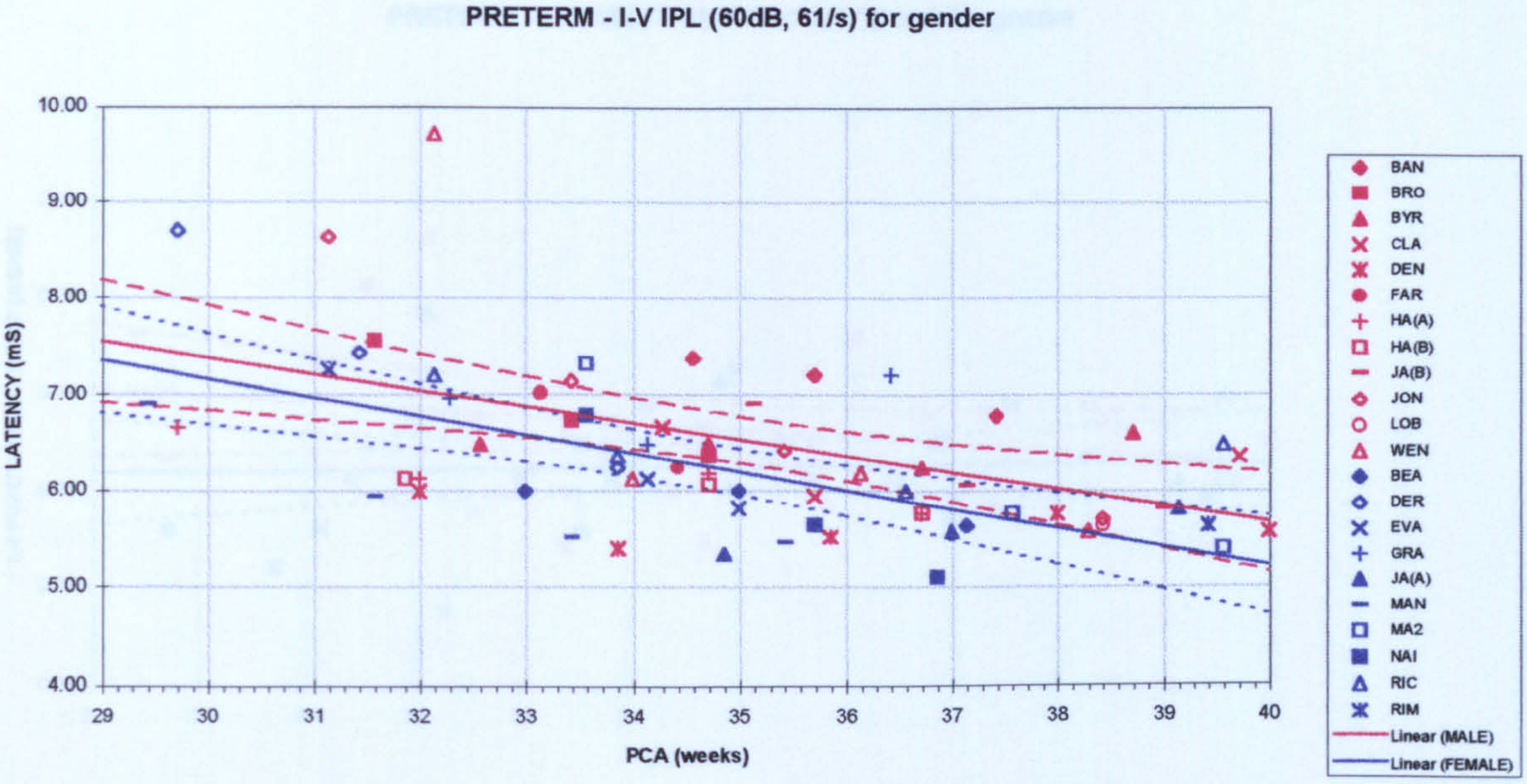


Female - r^2=0.40 n=30 P<0.001 Male - r^2=0.15 n=41 P<0.02

Figure D18 a/b

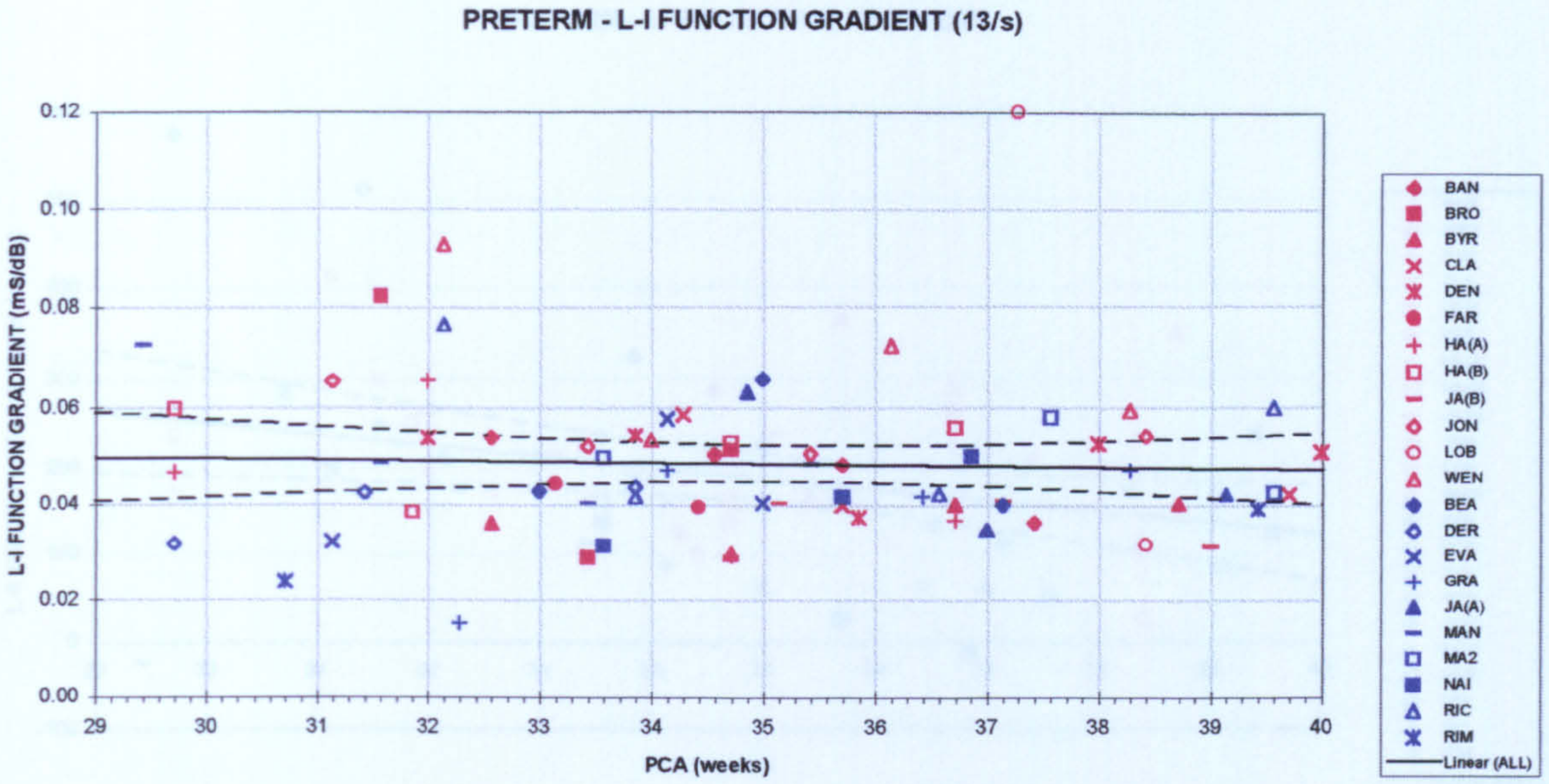


r^2=0.30 n=69 P<0.001

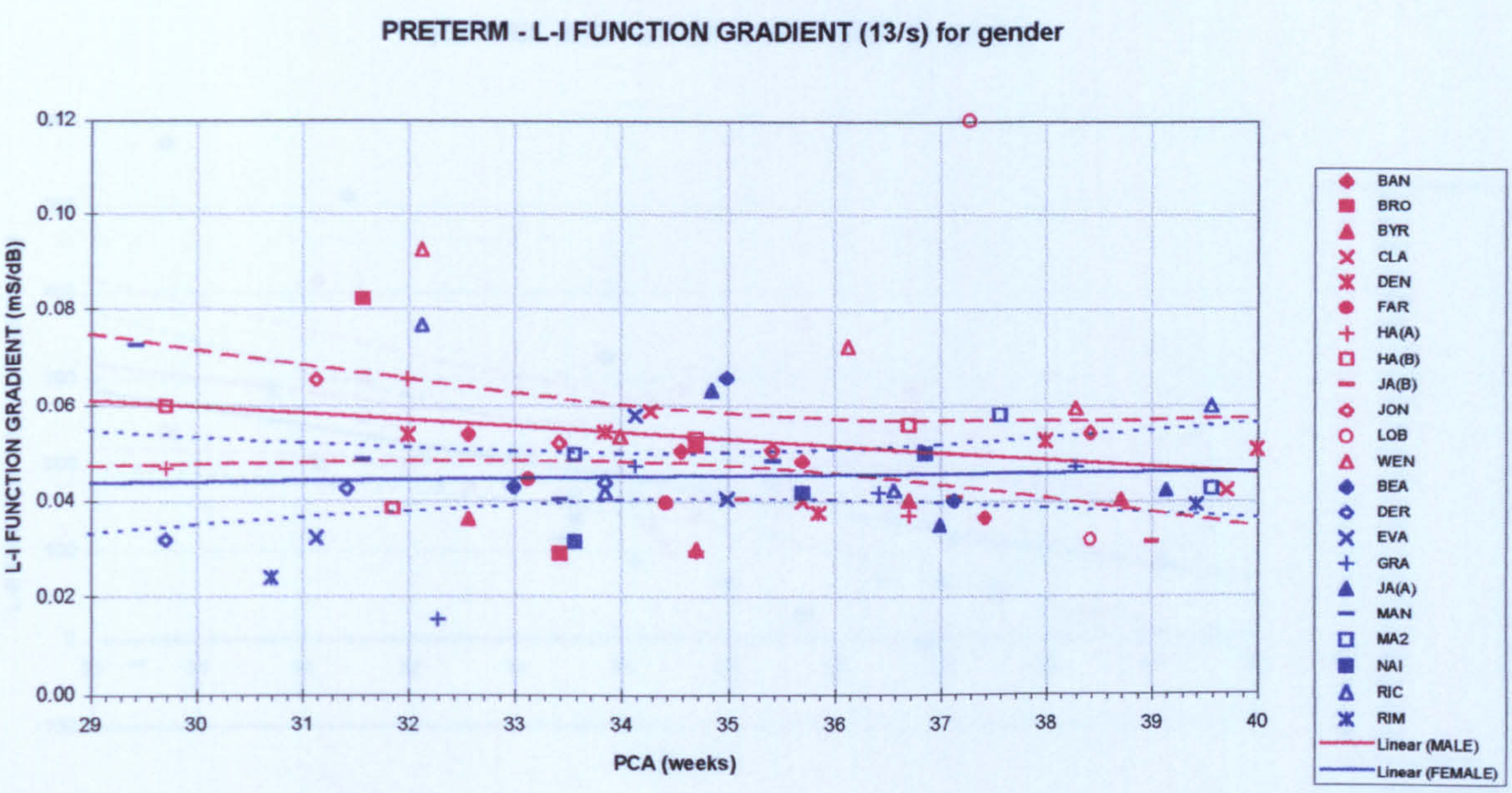


Female - r^2=0.40 n=30 P<0.001 Male - r^2=0.25 n=39 P<0.005

Figure D19 a/b

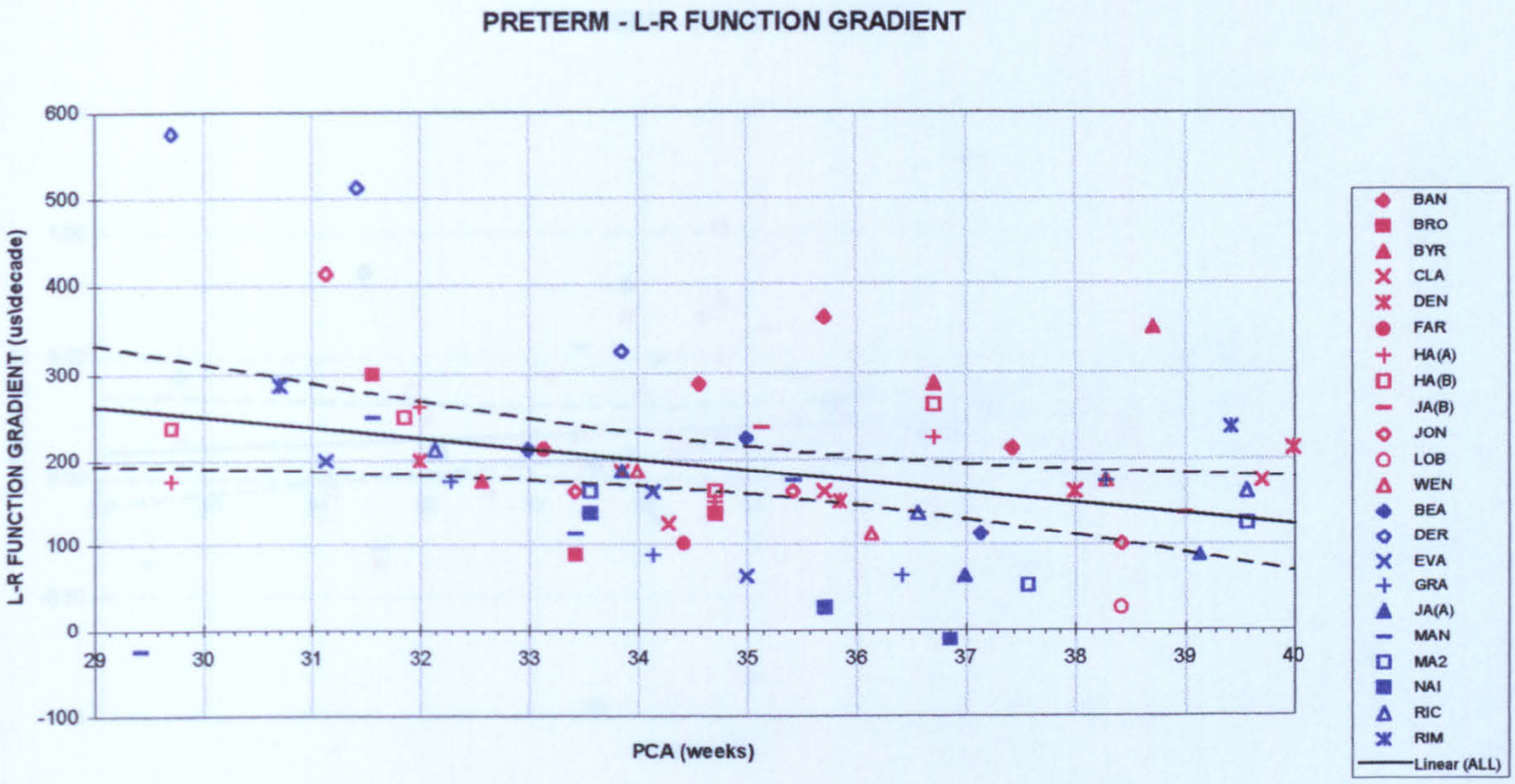


r^2=0.01 n=74 P>0.05

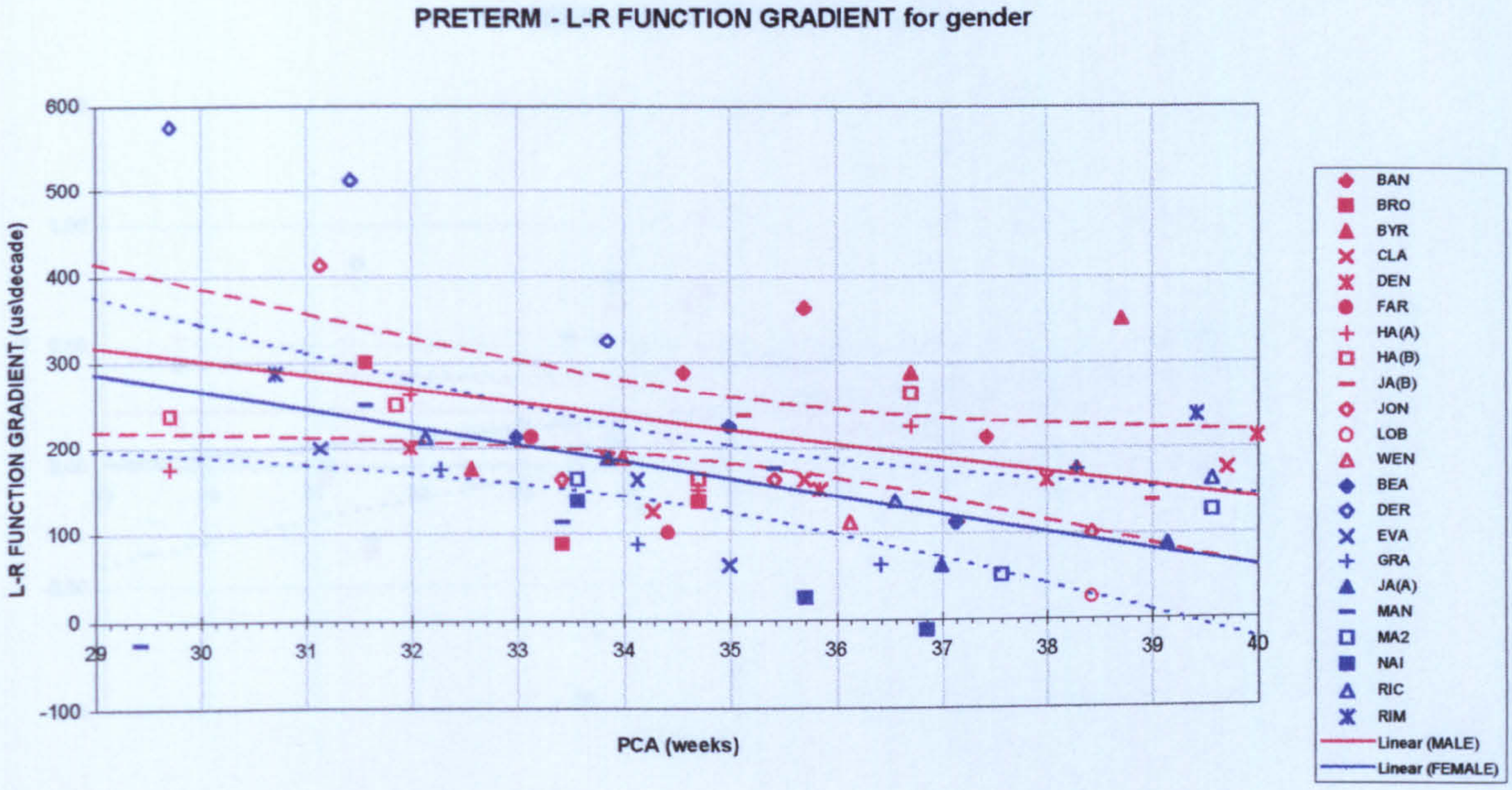


Female - r^2=0.01 n=32 P>0.05 Male - r^2=0.03 n=42 P>0.05

Figure D20 a/b

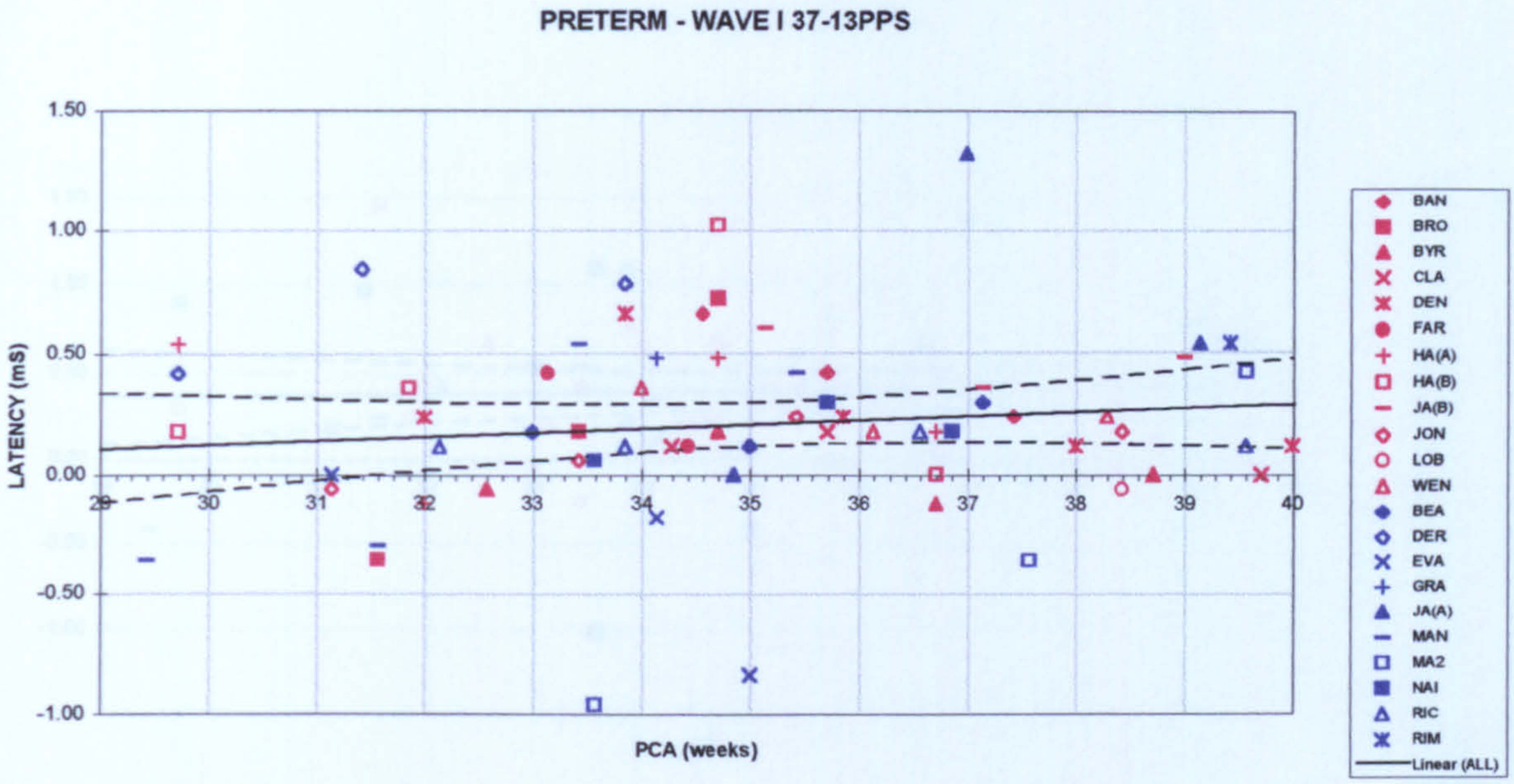


r^2=0.16 n=69 P<0.001

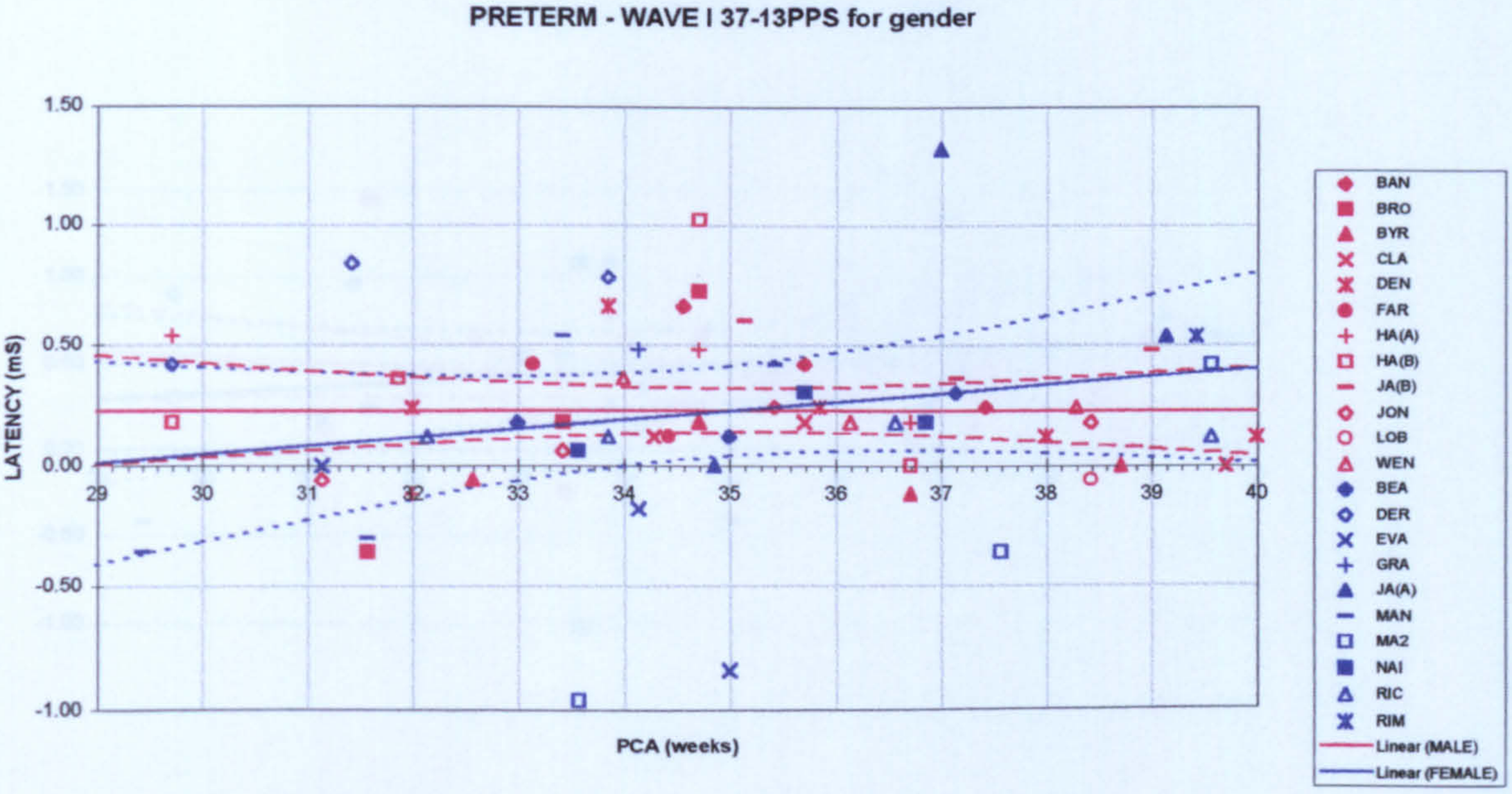


Female - r^2=0.33 n=29 P<0.005 Male - r^2=0.08 n=40 P>0.05

Figure D21 a/b

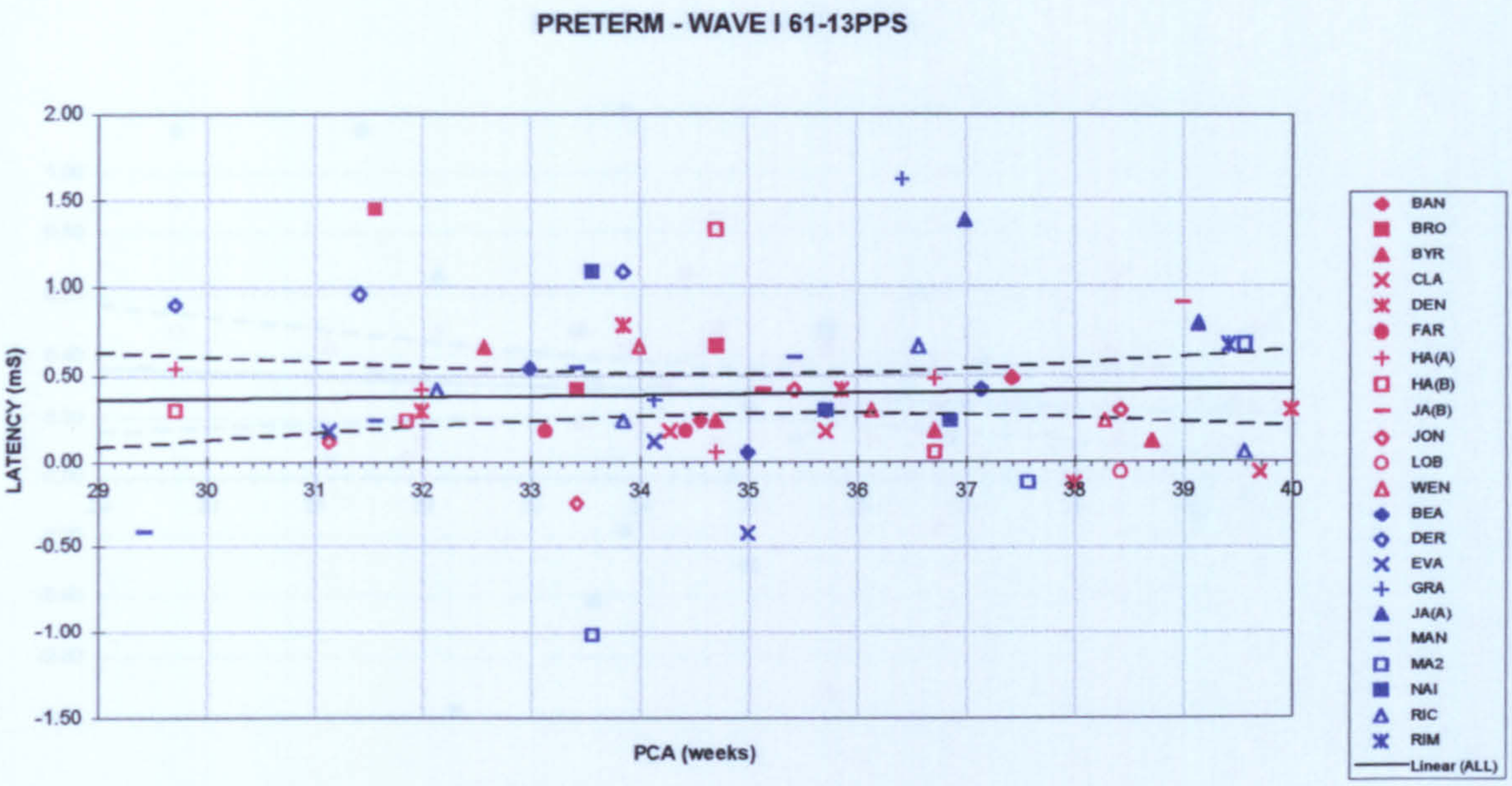


r^2=0.01 n=67 P>0.05

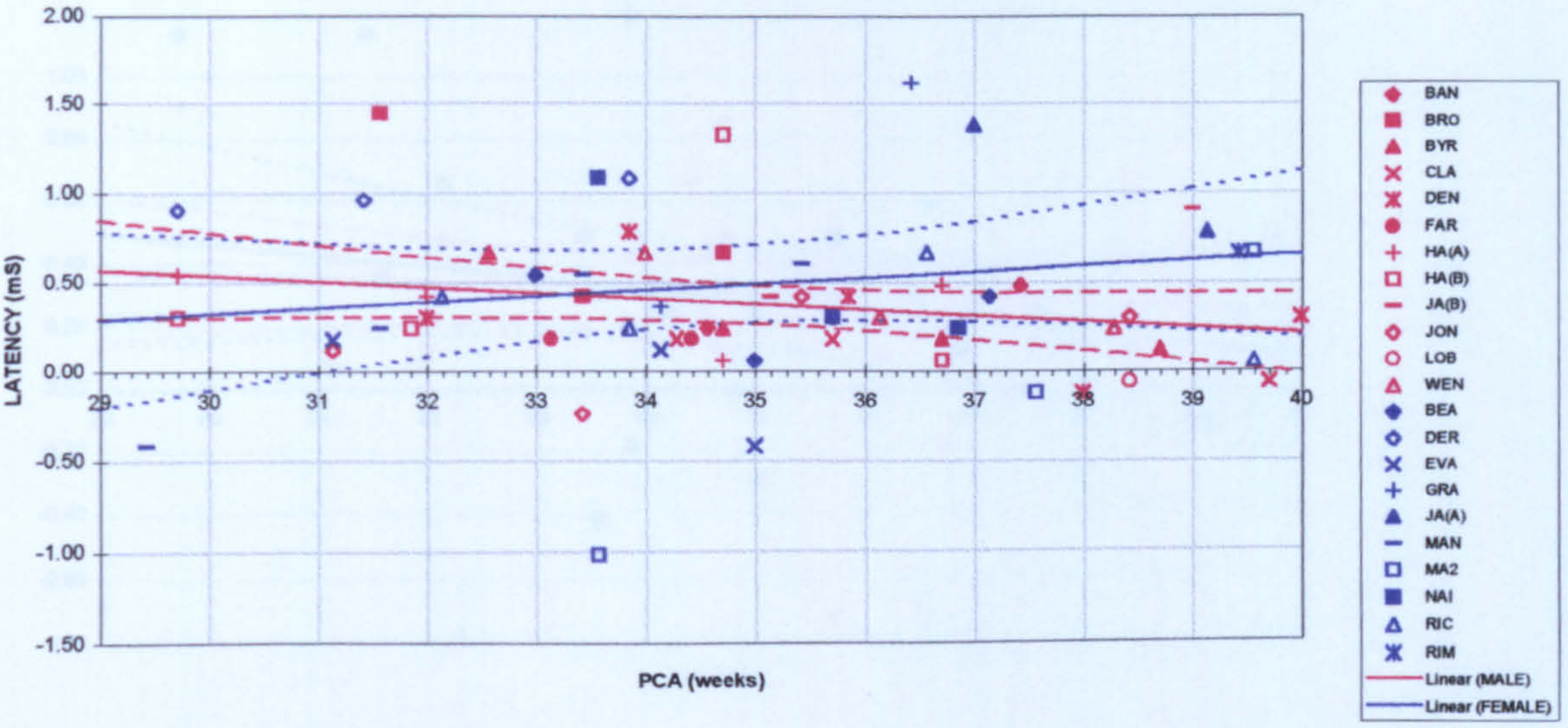


Female - r^2=0.05 n=28 P>0.05 Male - r^2=0.00 n=39 P>0.05

Figure D22 a/b

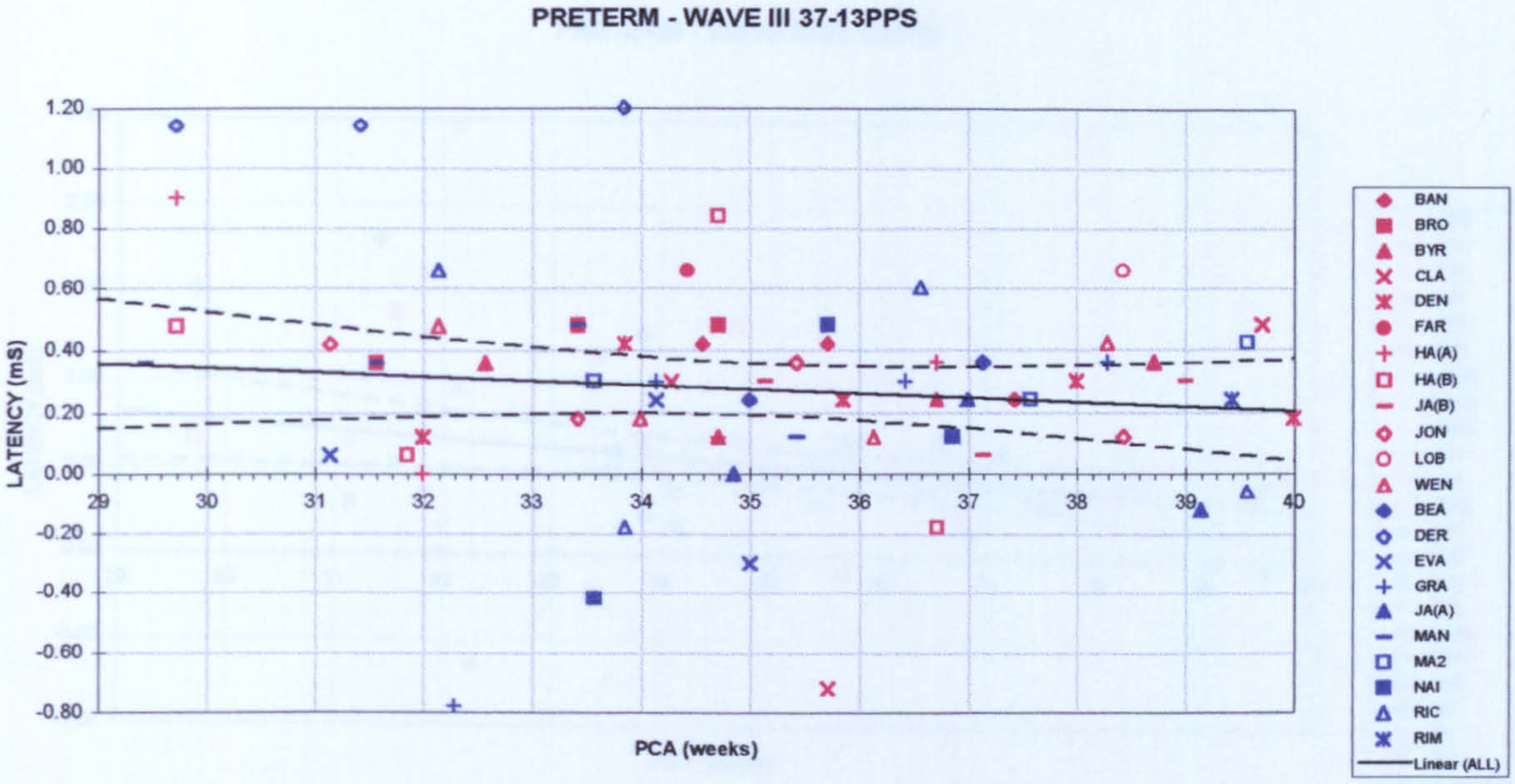


r^2=0.00 n=67 P>0.05

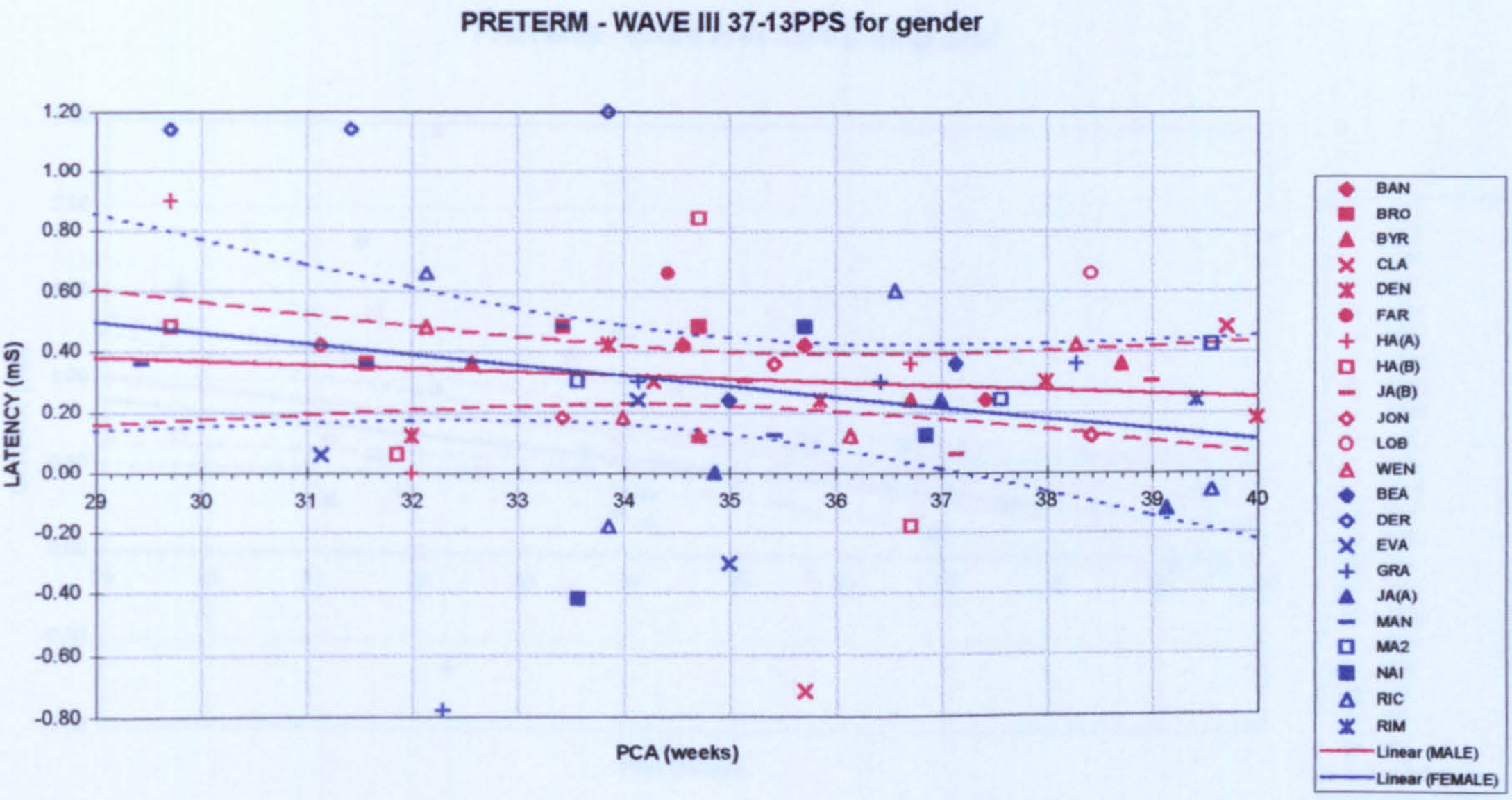


Female - r^2=0.02 n=28 P>0.05 Male - r^2=0.06 n=39 P>0.05

Figure D23 a/b

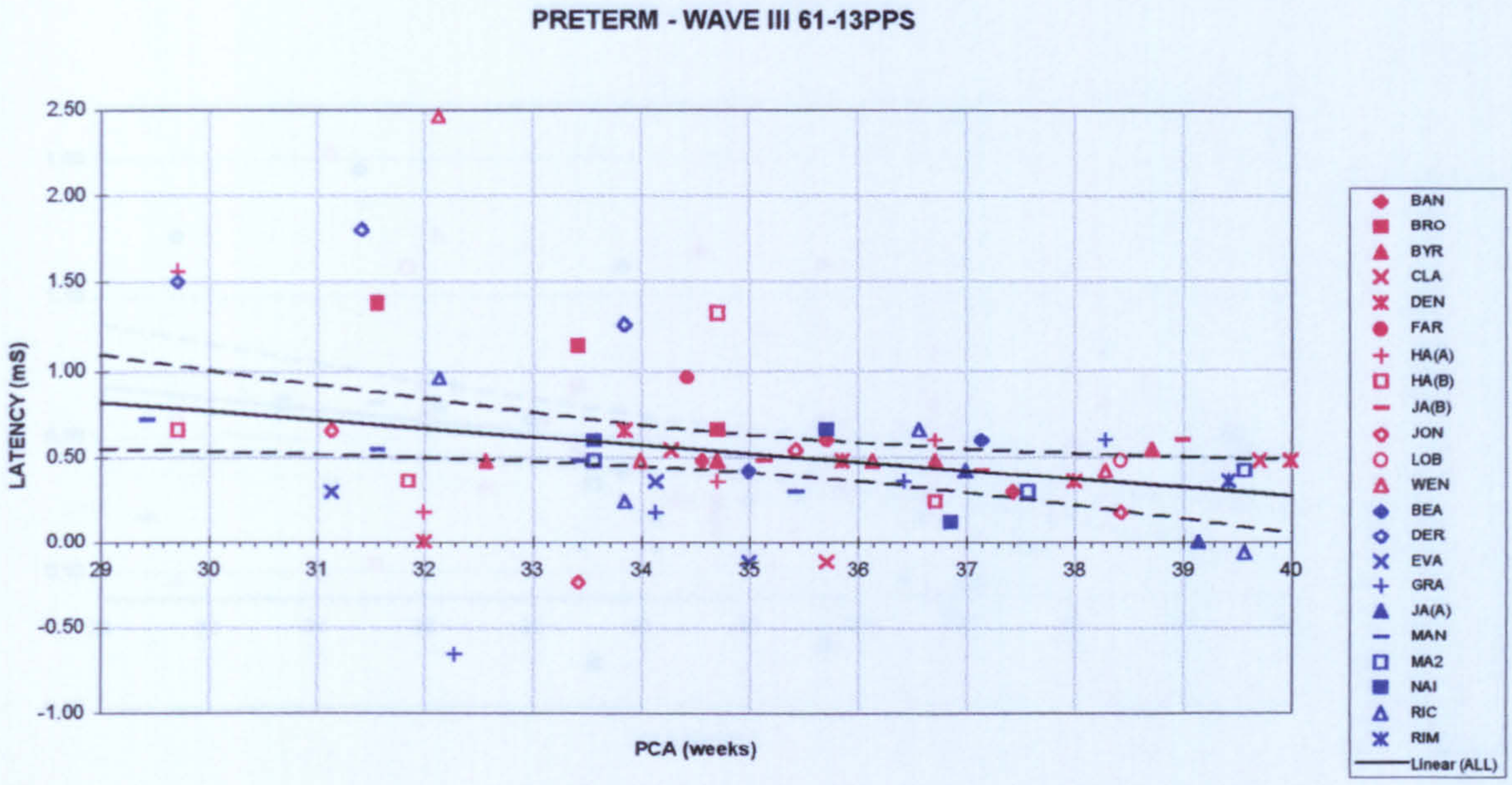


r^2=0.04 n=69 P>0.05

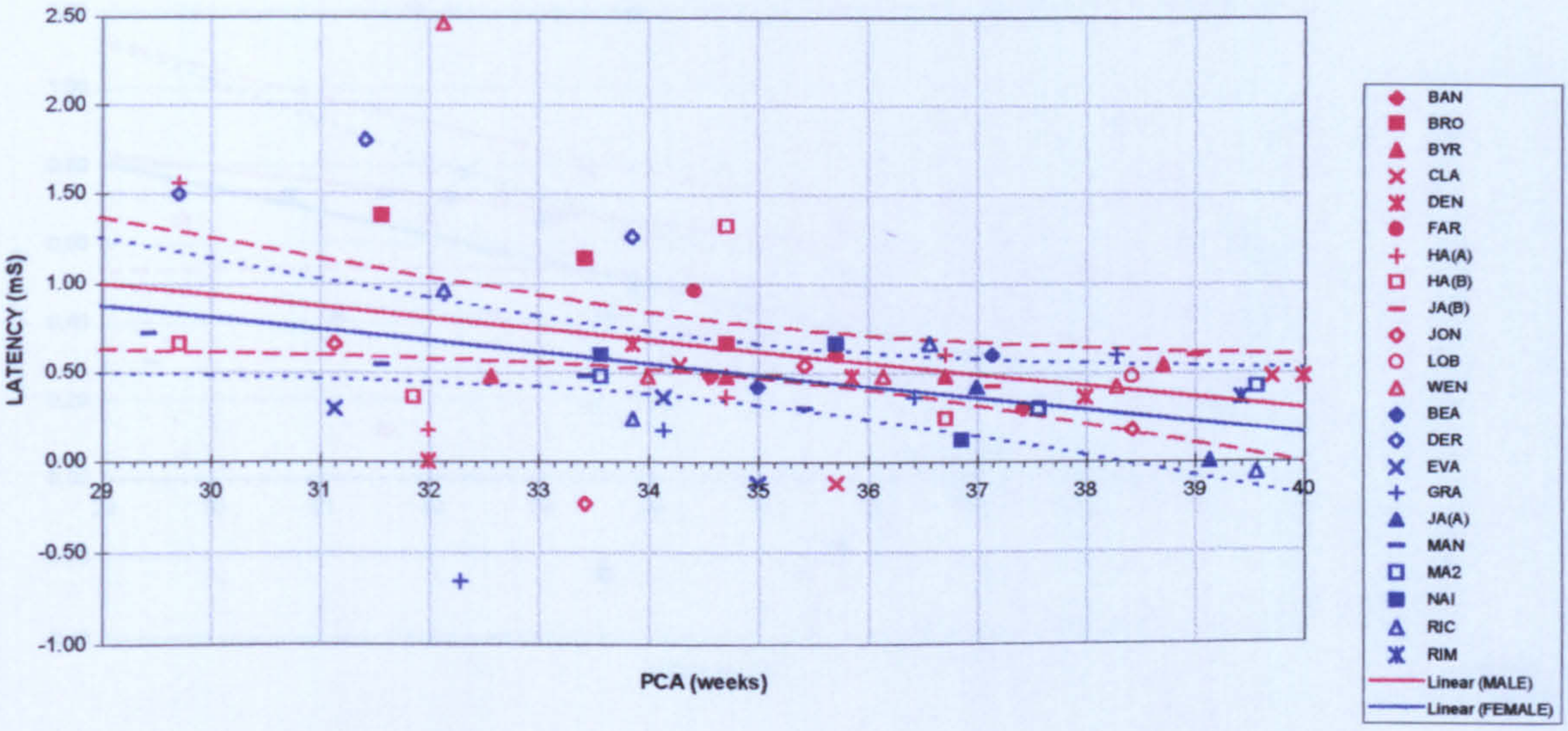


Female - r^2=0.05 n=30 P>0.05 Male - r^2=0.03 n=39 P>0.05

Figure D24 a/b

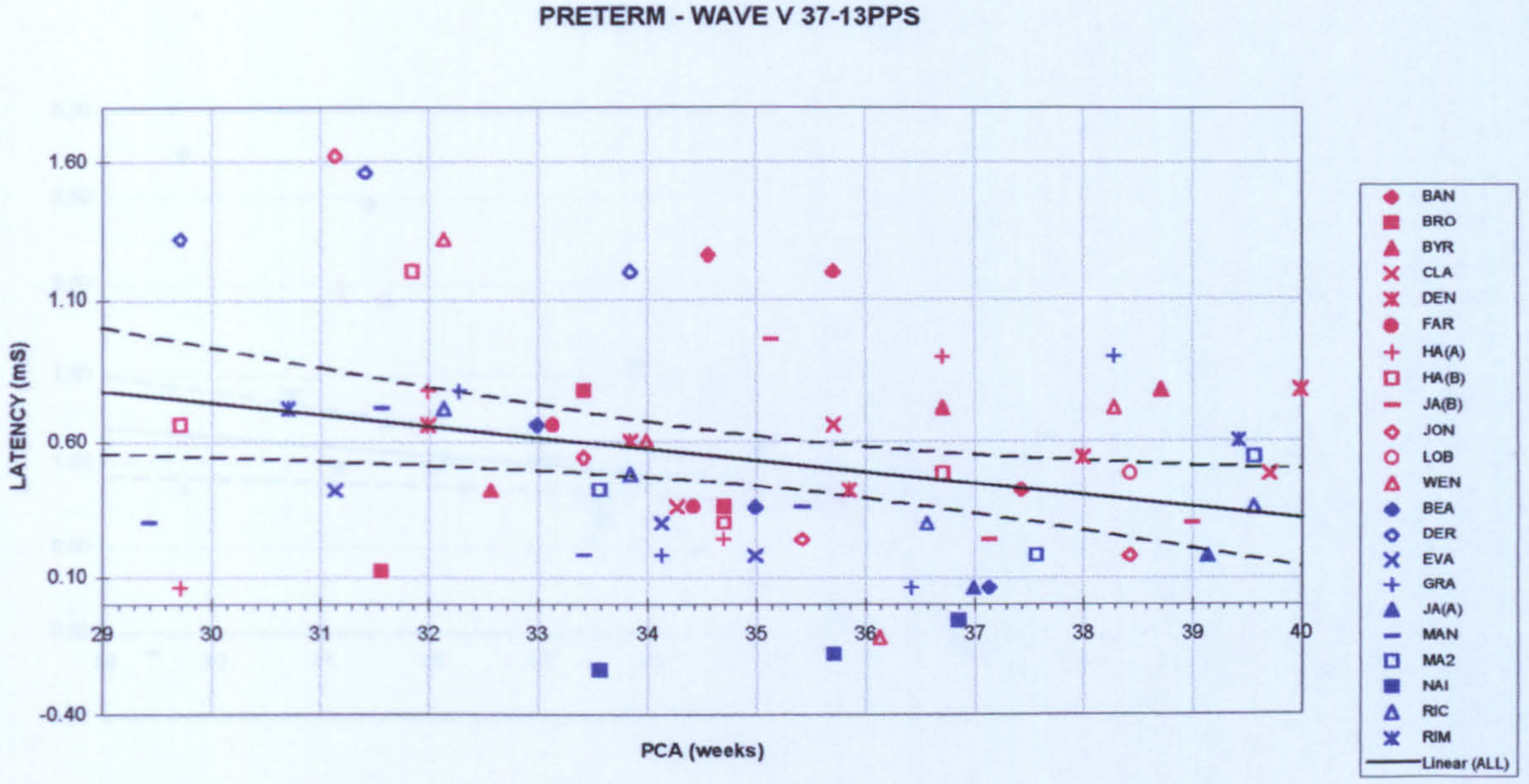


r^2=0.13 n=68 P<0.005

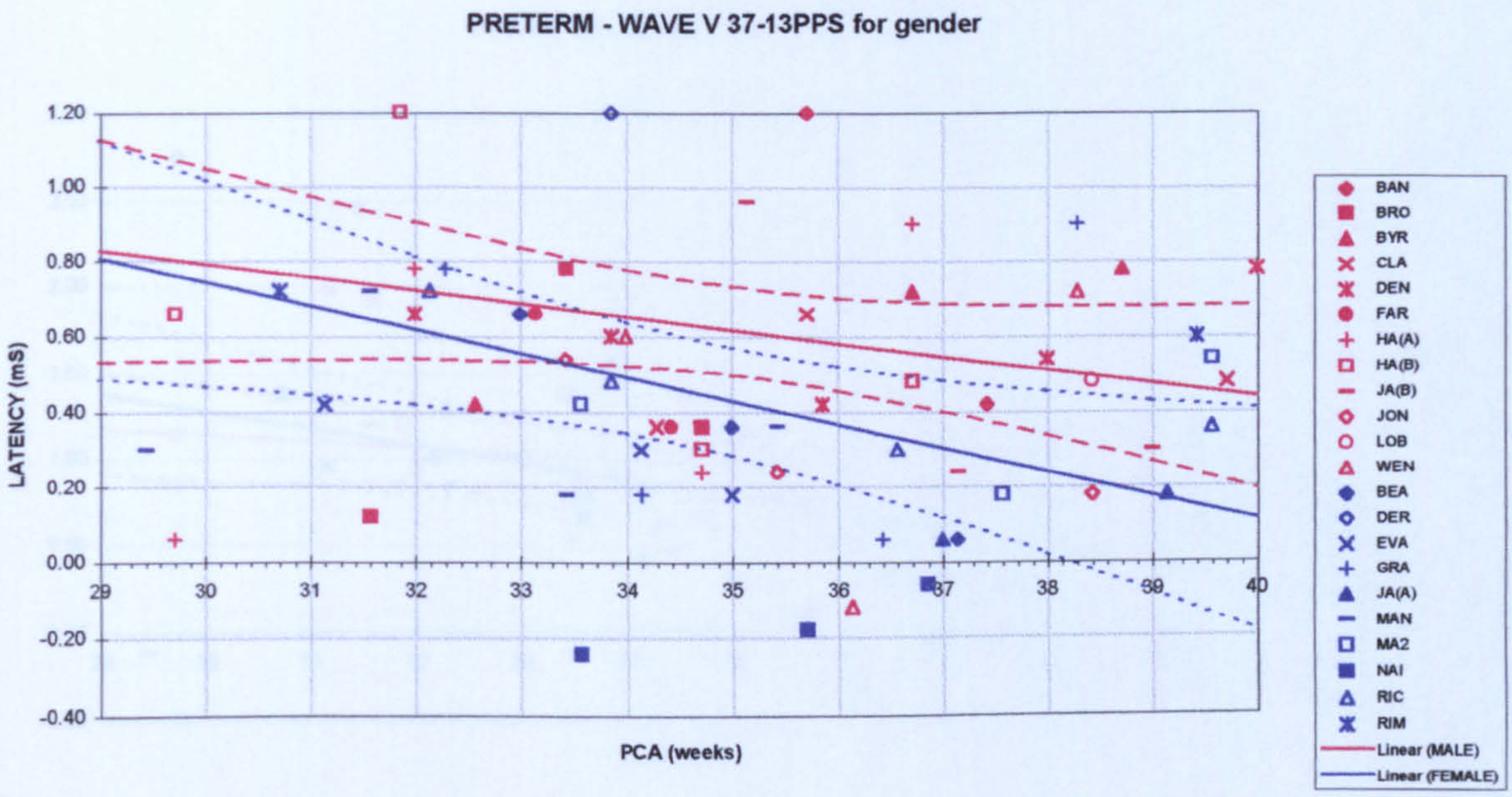


Female - r^2=0.16 n=29 P<0.005 Male - r^2=0.12 n=39 P<0.05

Figure D25 a/b

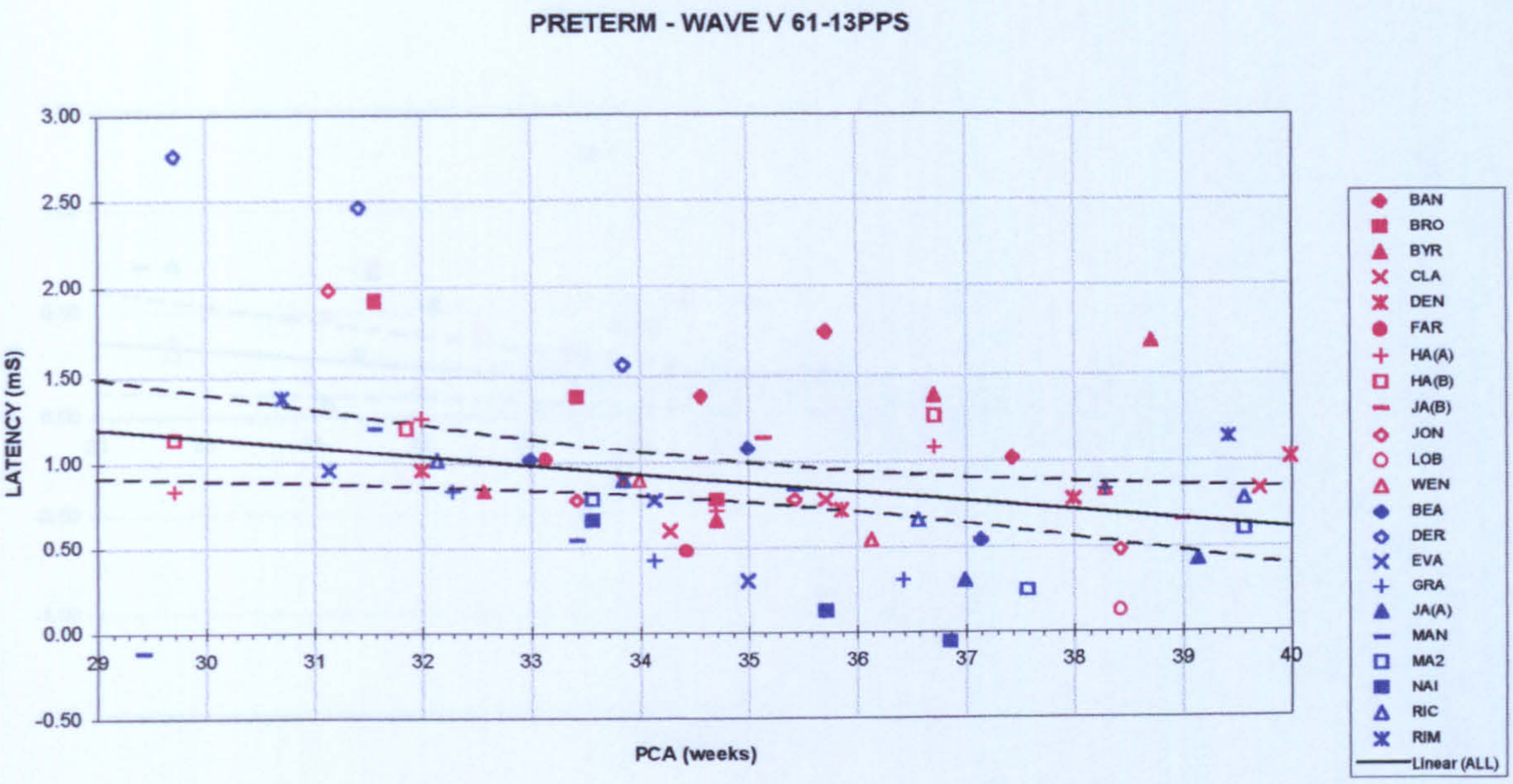


r^2=0.07 n=71 P<0.025

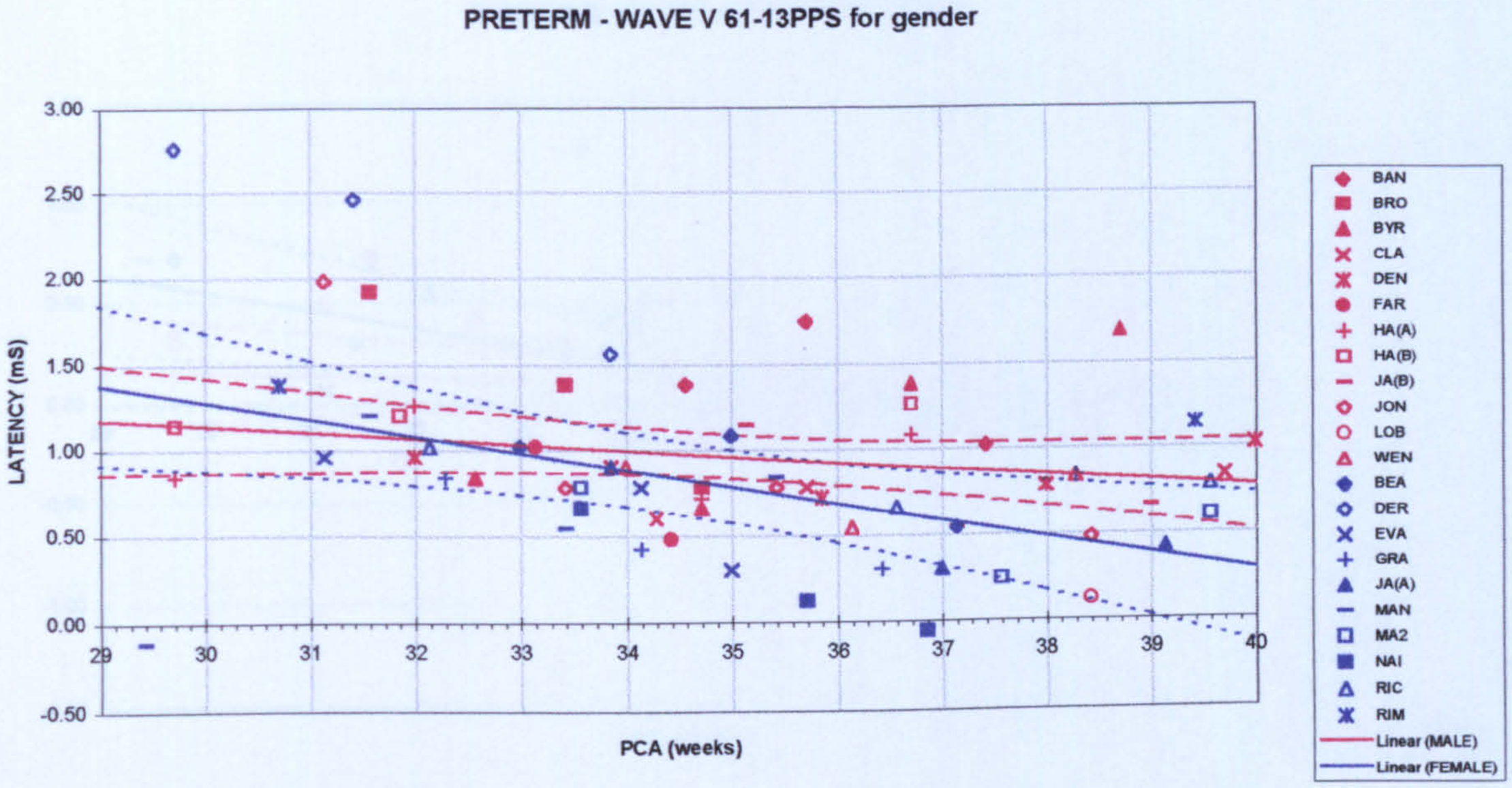


Female - r^2=0.16 n=31 P<0.05 Male - r^2=0.03 n=40 P>0.05

Figure D26 a/b

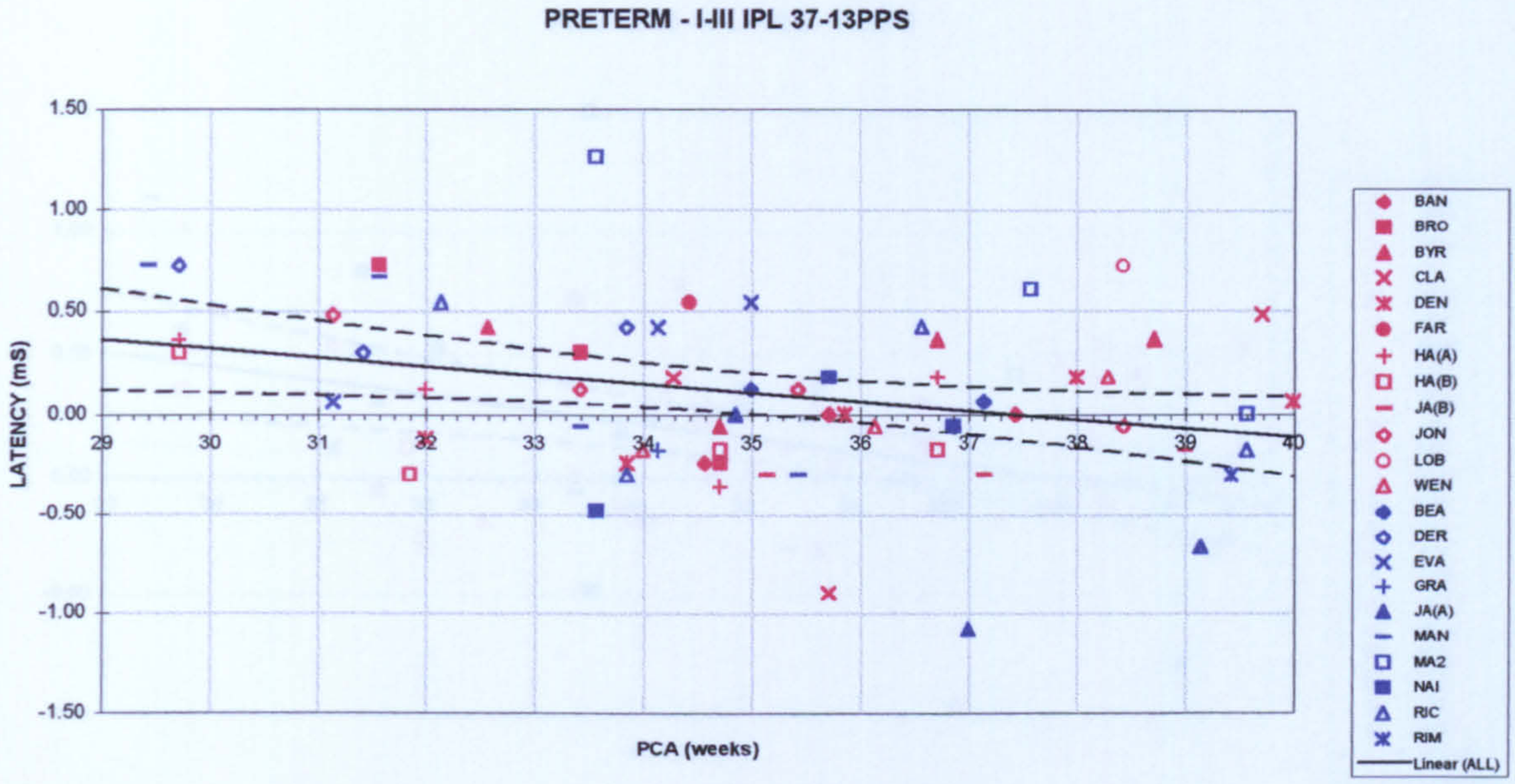


r^2=0.13 n=70 P<0.005

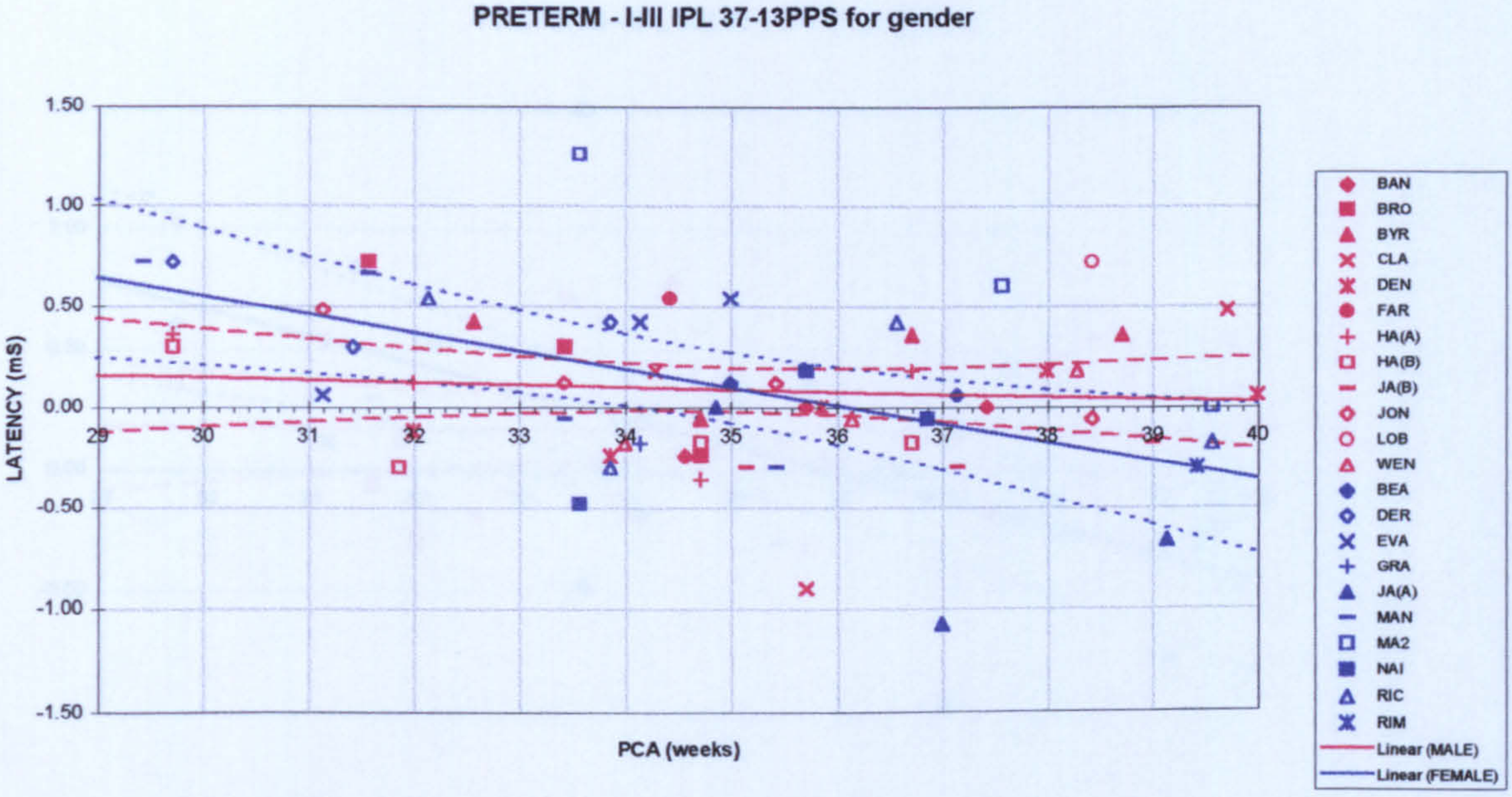


Female - r^2=0.19 n=31 P<0.02 Male - r^2=0.10 n=39 P>0.05

Figure D27 a/b

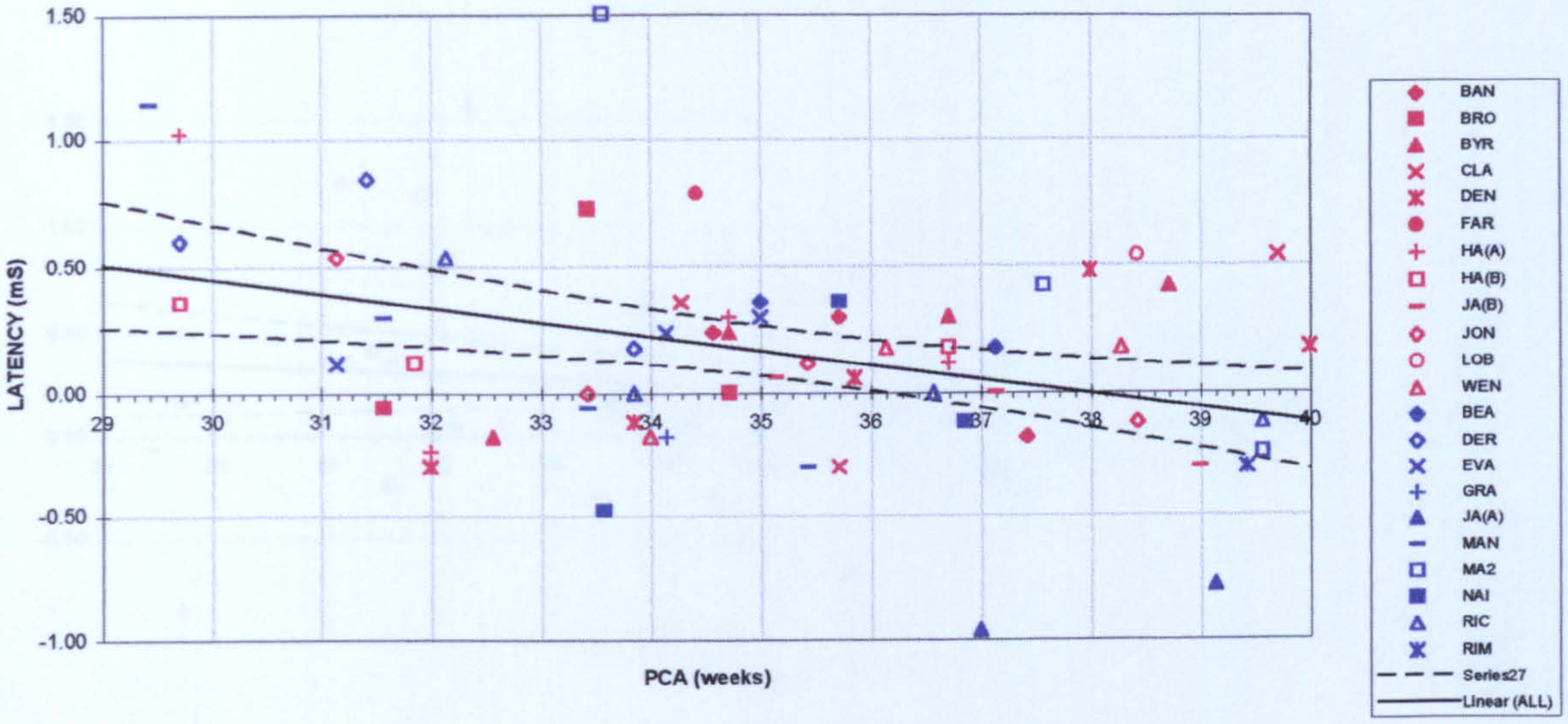


r^2=0.09 n=65 P<0.02

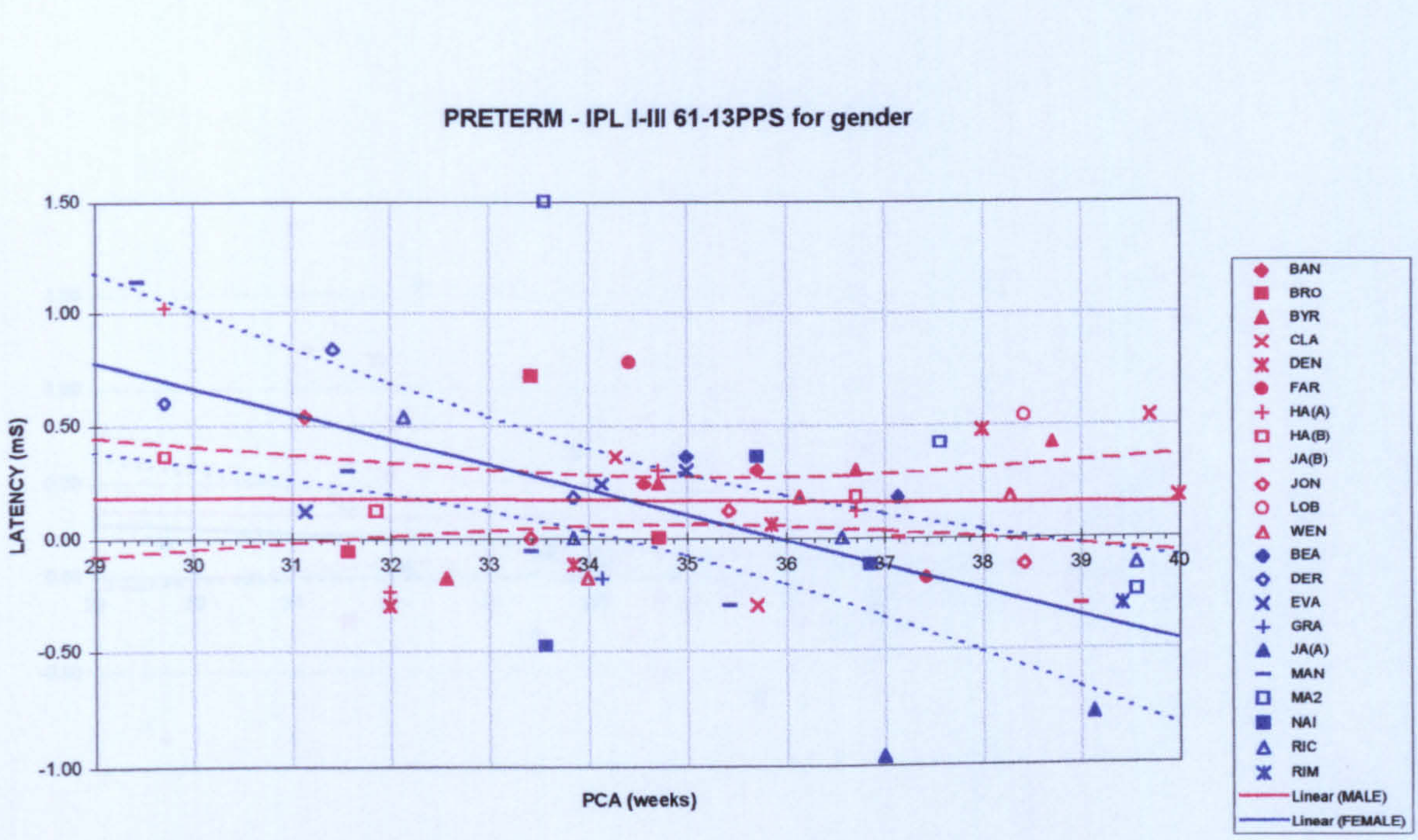


Female - r^2=0.25 n=27 P<0.01 Male - r^2=0.01 n=38 P>0.05

Figure D28 a/b

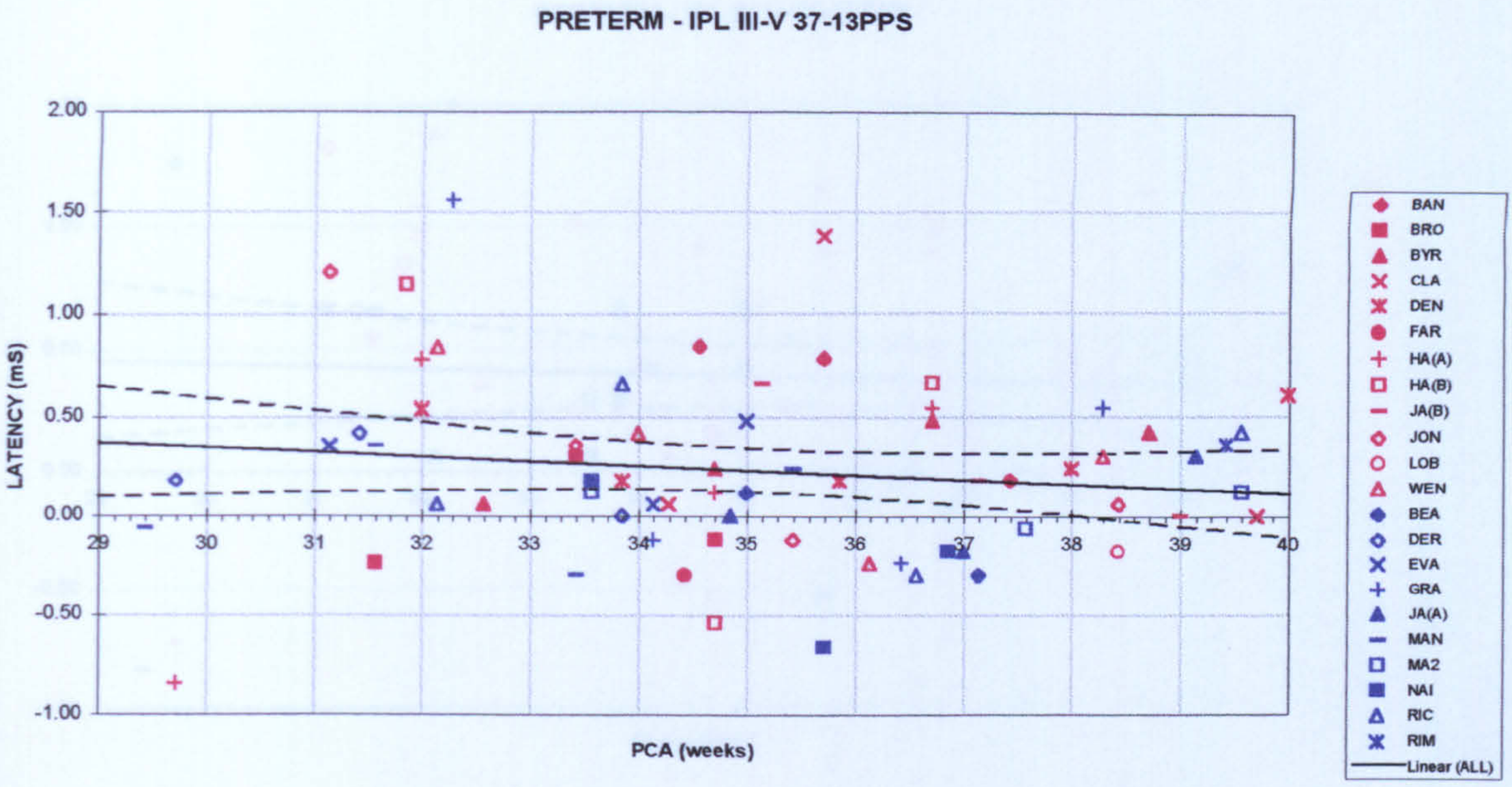


r^2=0.12 n=64 P<0.005

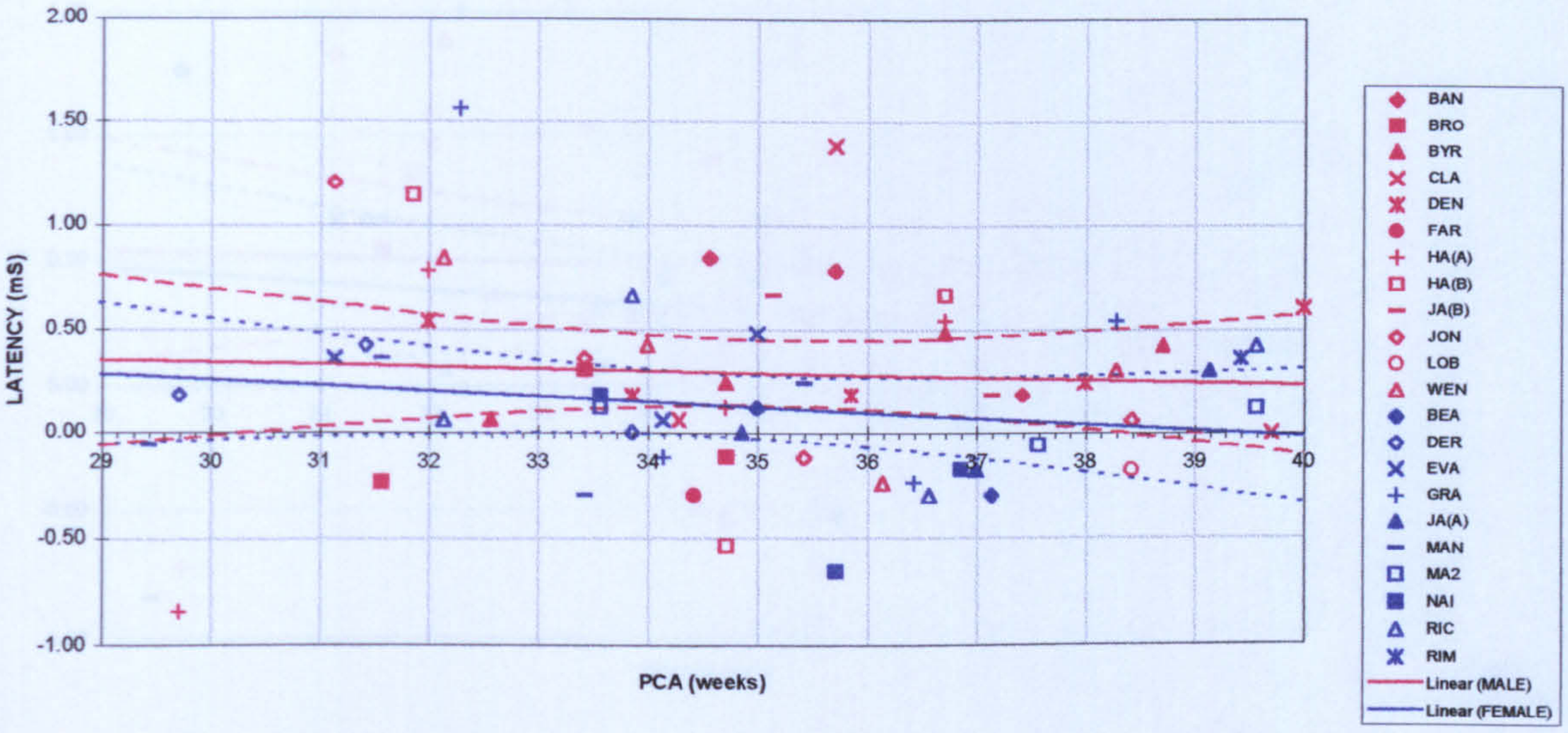


Female - r^2=0.35 n=26 P<0.005 Male - r^2=0.00 n=38 P>0.05

Figure D29 a/b

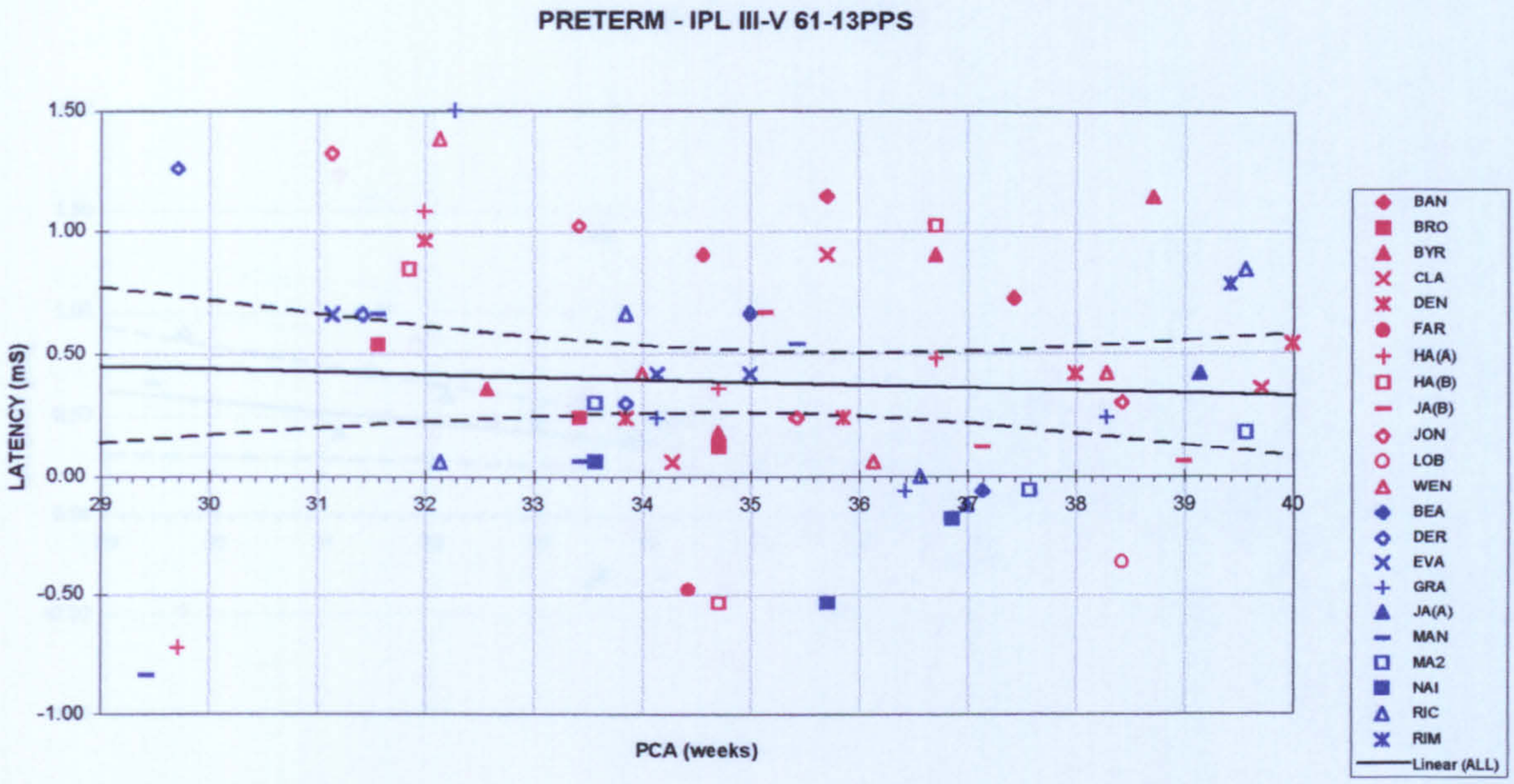


r^2=0.01 n=68 P>0.05

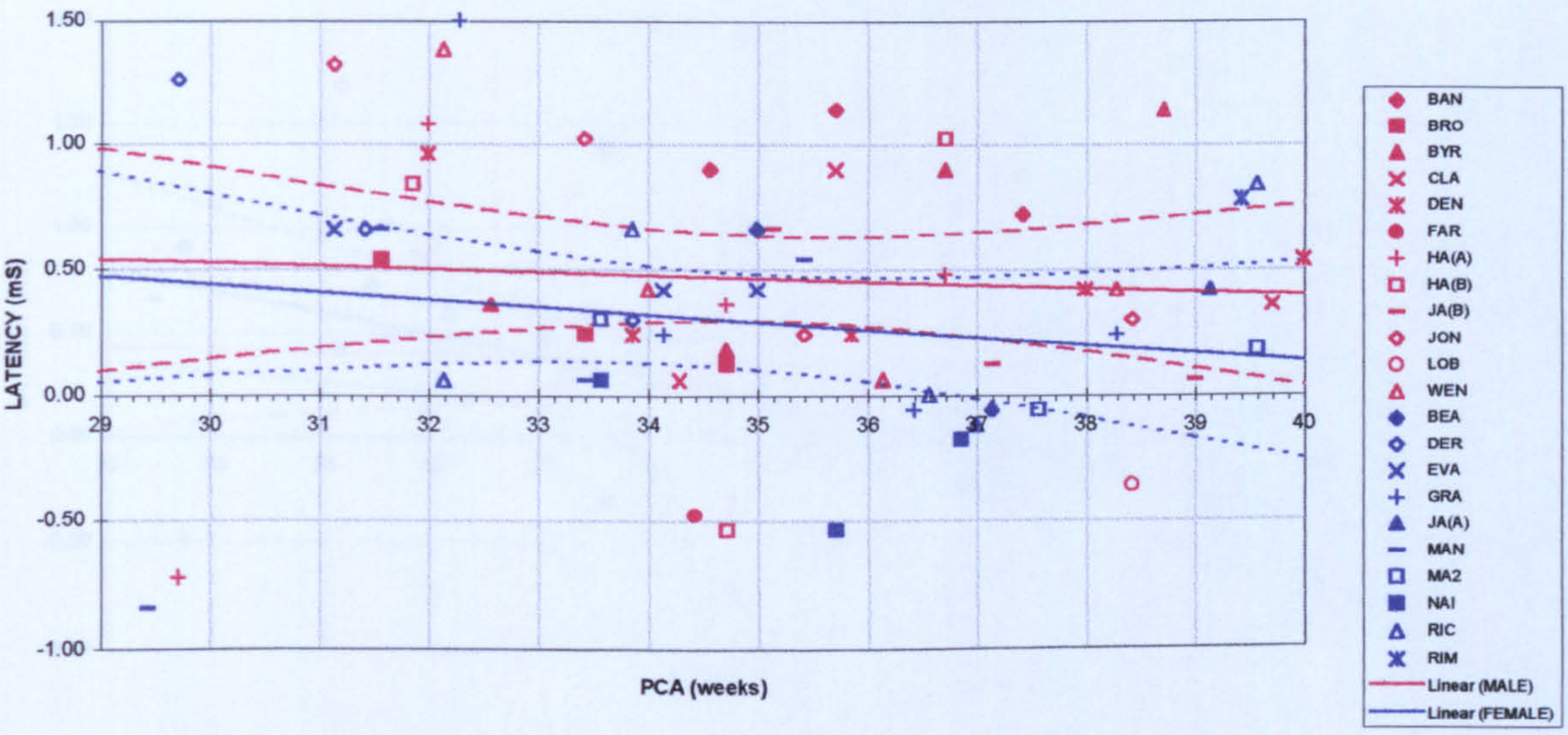


Female - r^2=0.02 n=30 P>0.05 Male - r^2=0.00 n=38 P>0.05

Figure D30 a/b

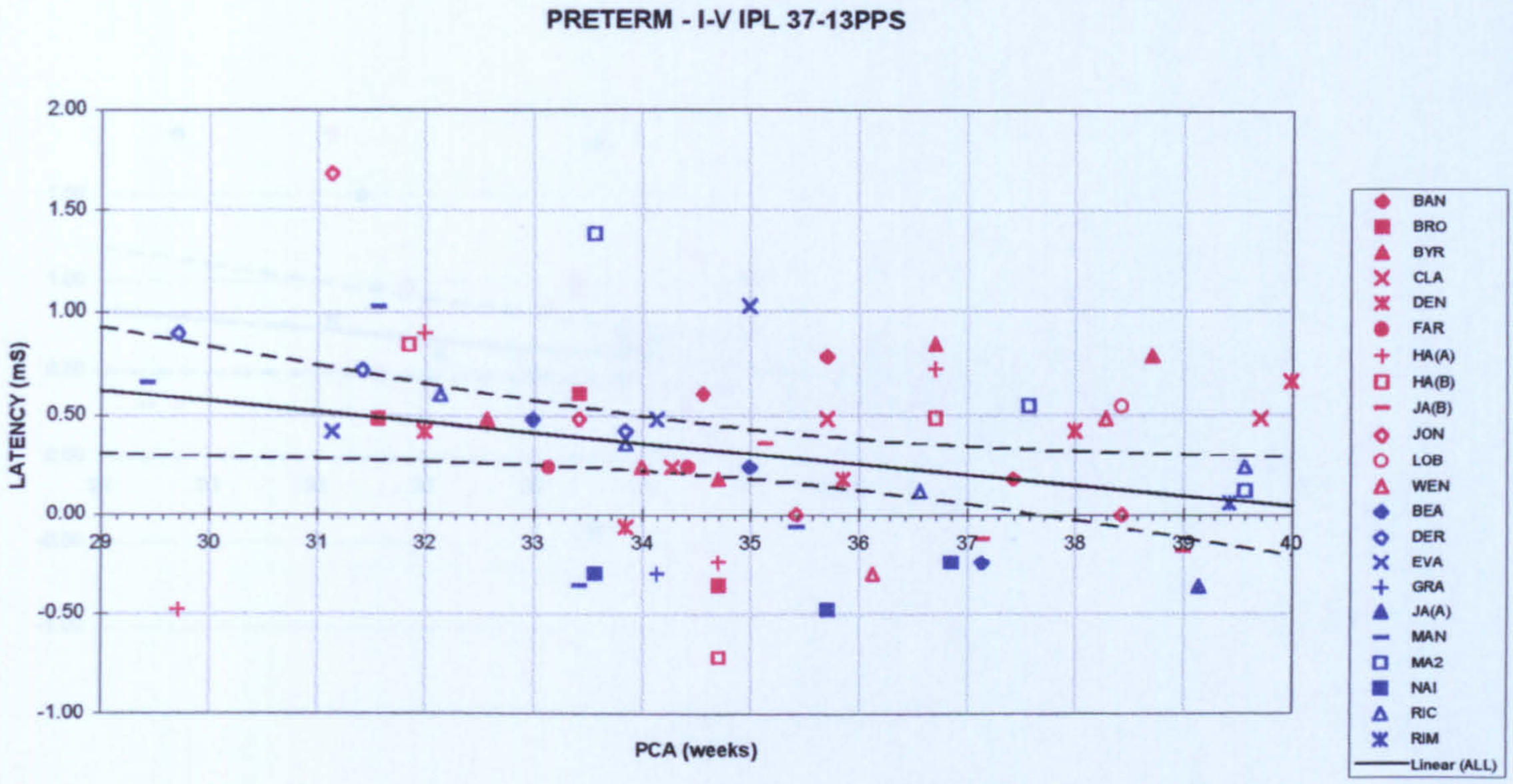


r^2=0.01 n=67 P>0.05

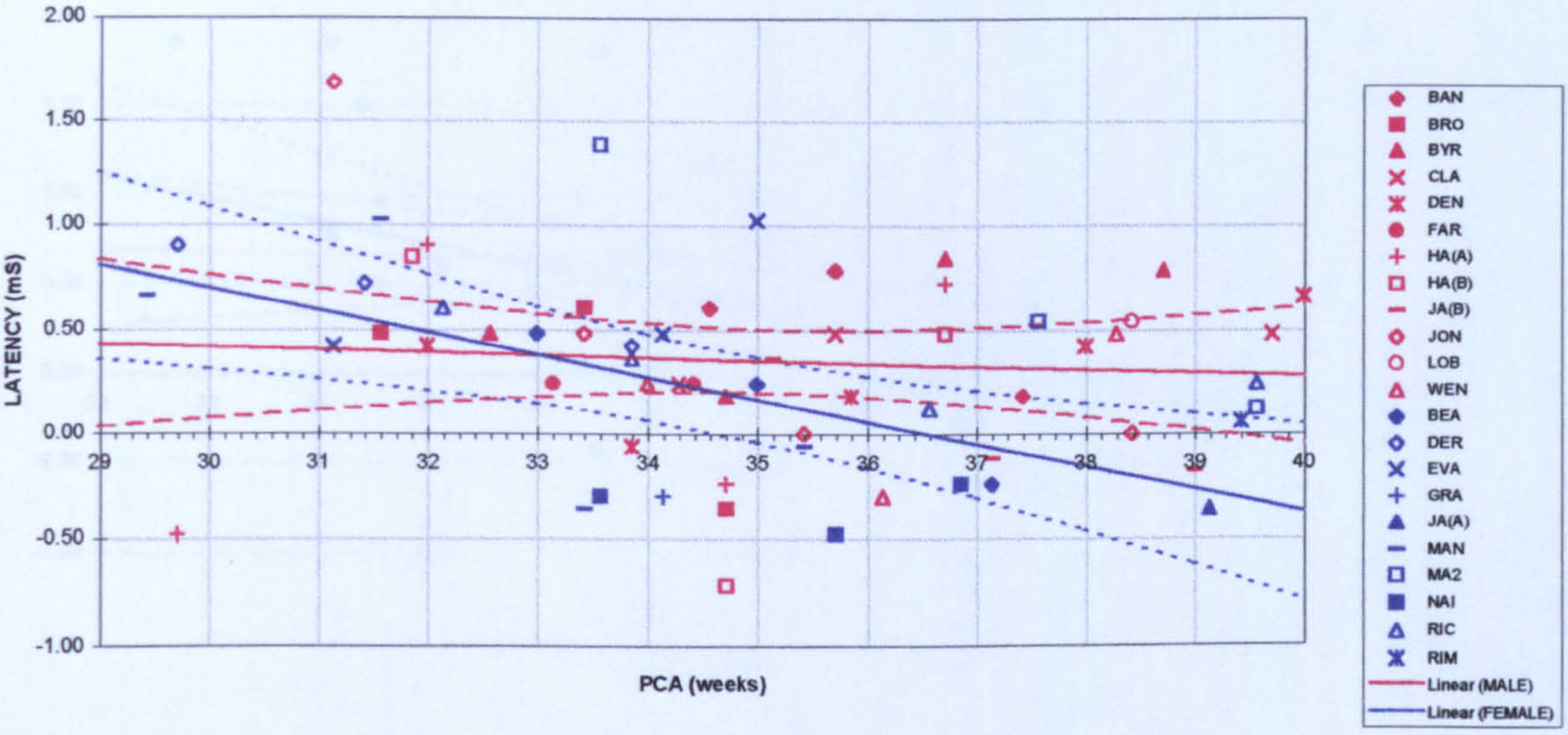


Female - r^2=0.02 n=29 P>0.05 Male - r^2=0.01 n=38 P>0.05

Figure D31 a/b

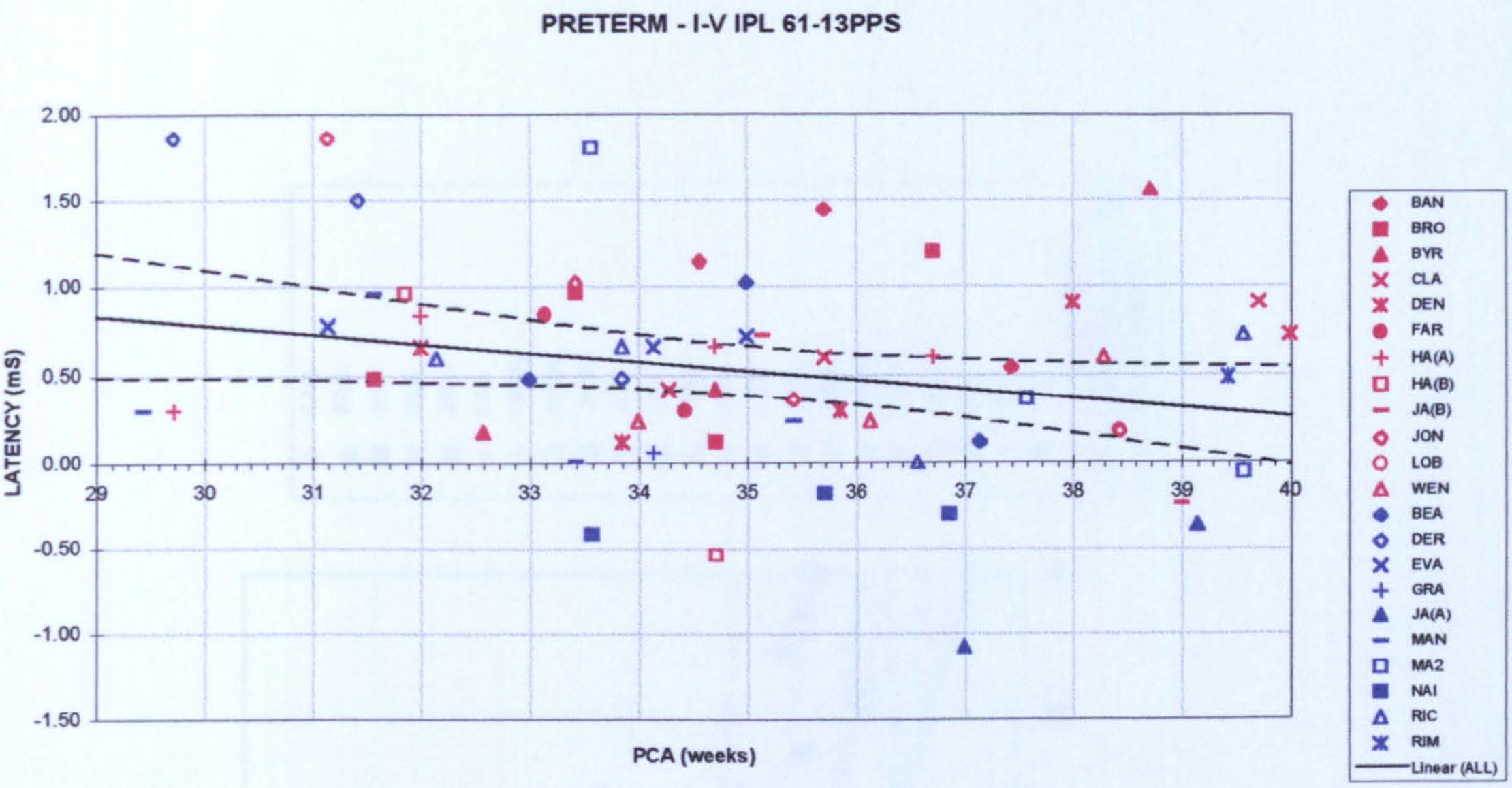


r^2=0.07 n=65 P<0.05

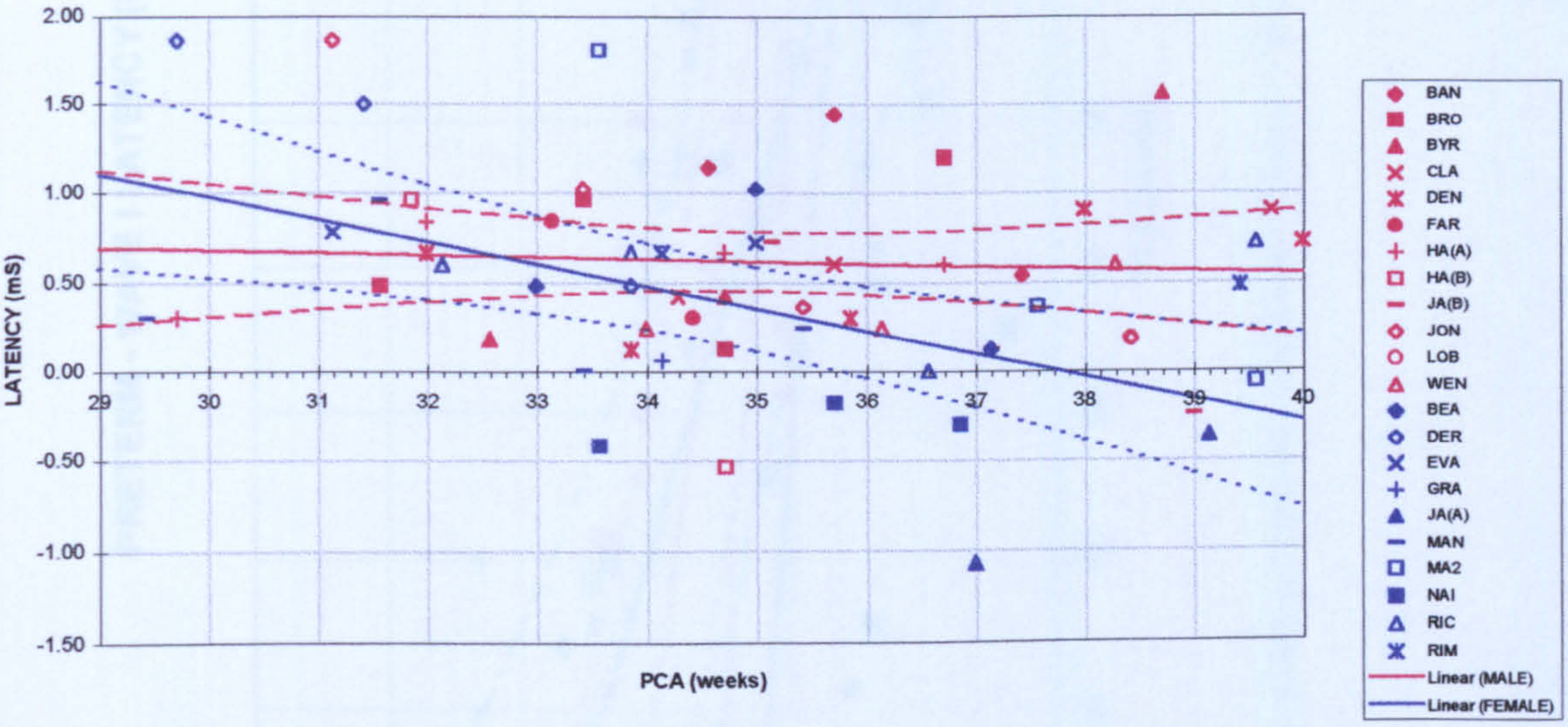


Female - r^2=0.24 n=27 P<0.01 Male - r^2=0.00 n=38 P>0.05

Figure D32 a/b

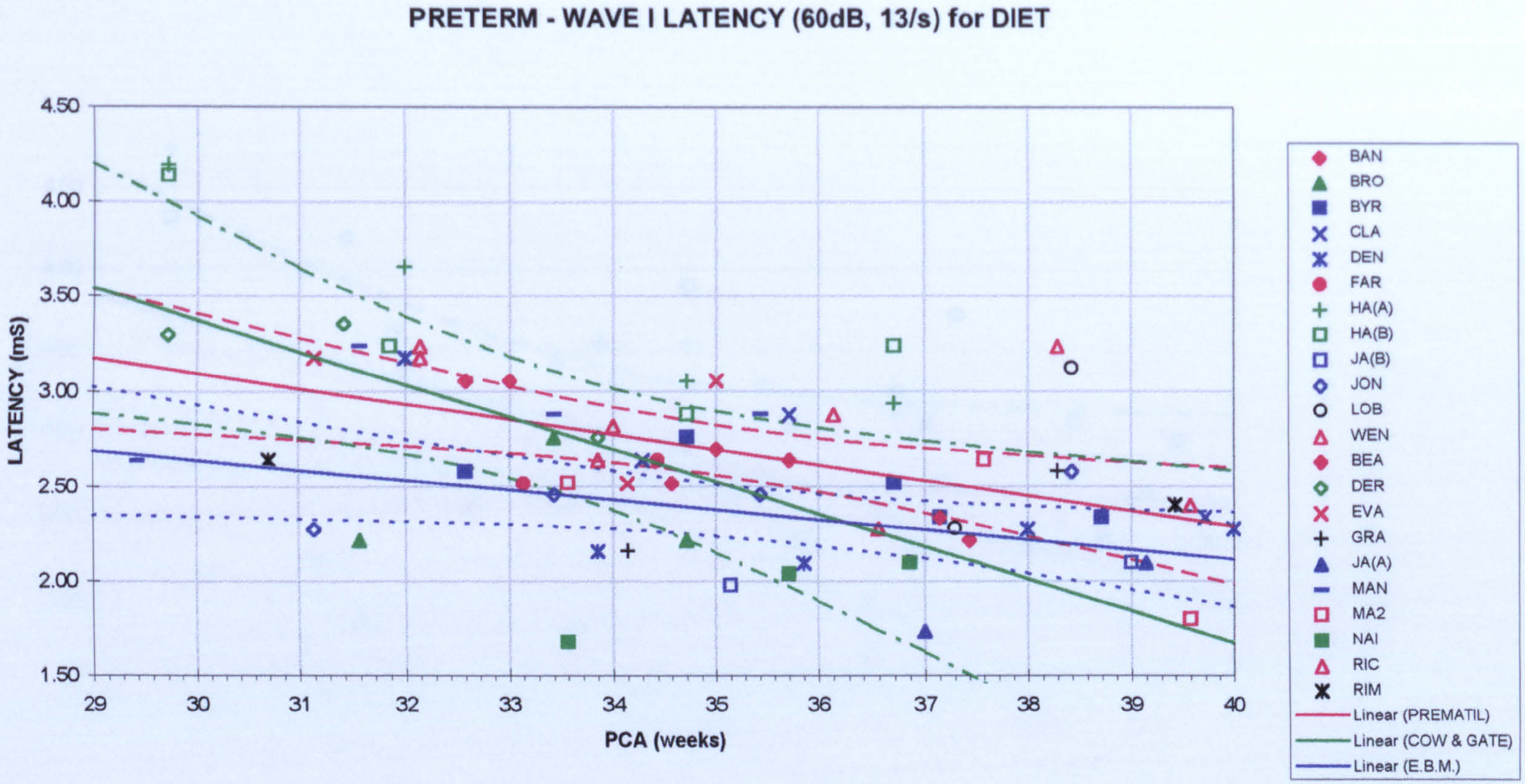


r^2=0.07 n=65 P<0.05



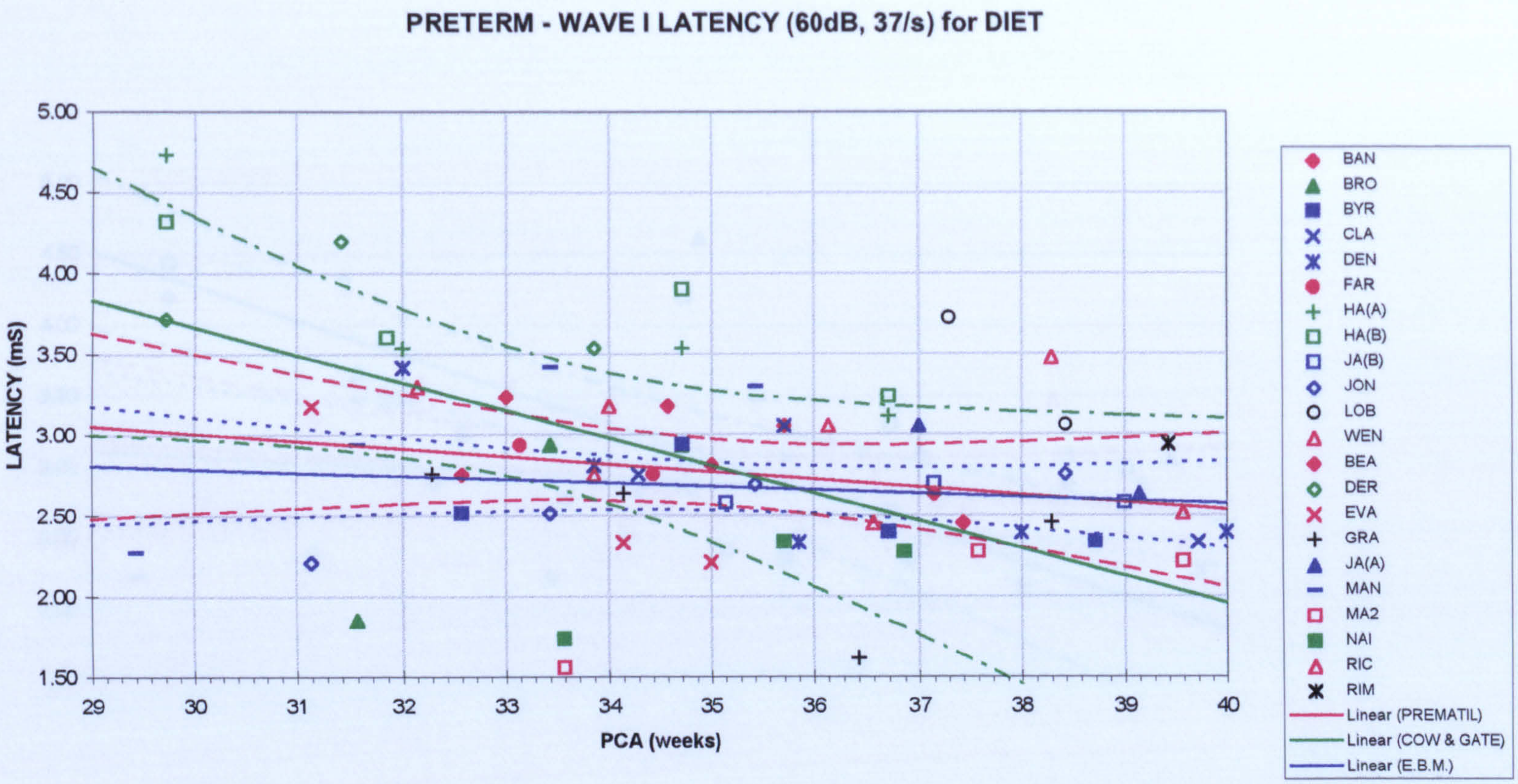
Female - r^2=0.25 n=27 P<0.01 Male - r^2=0.00 n=38 P>0.05

Figure D1c



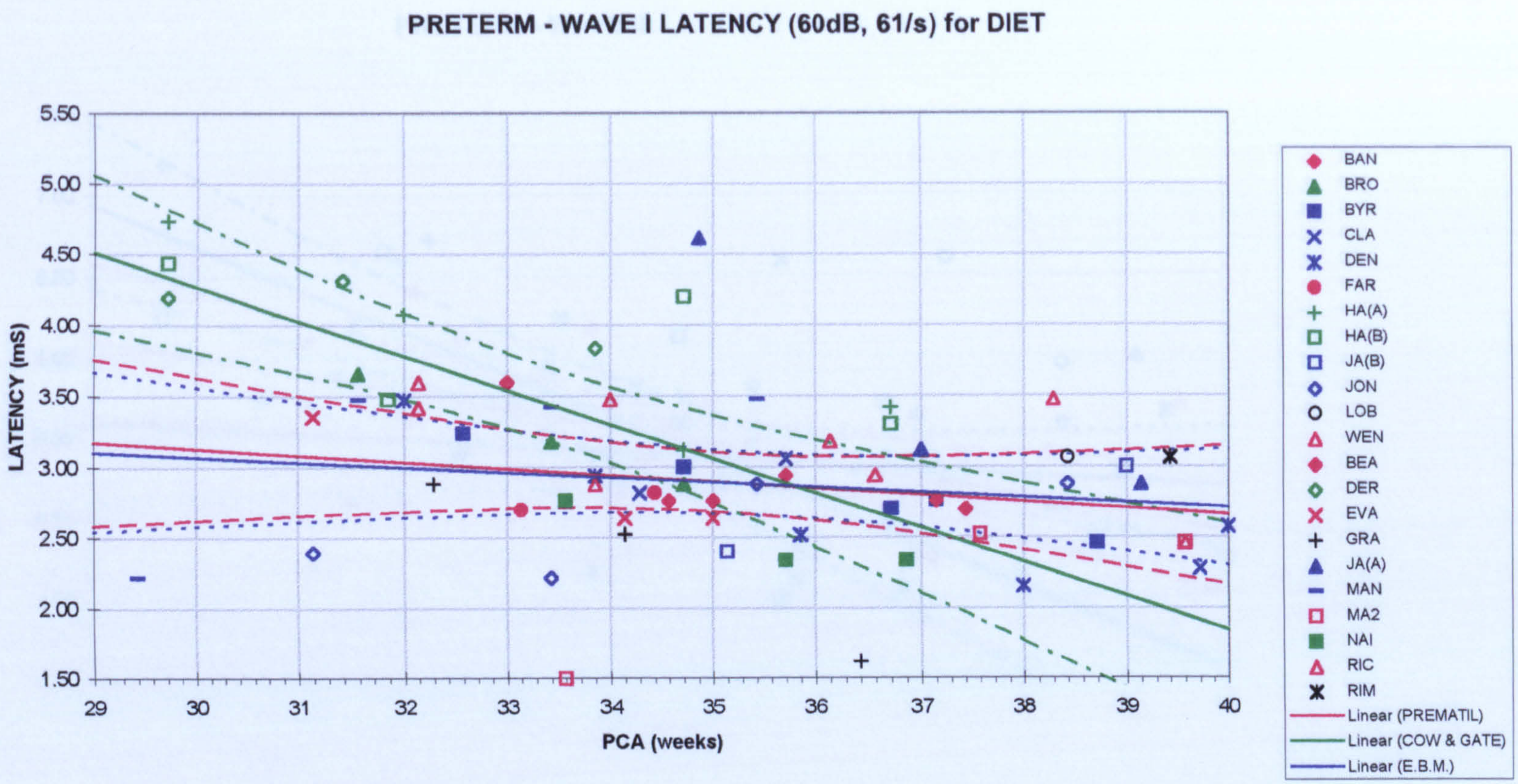
E.B.M. - r^2=0.24 n=25 P<0.02 Prematil - r^2=0.37 n=23 P<0.005 Cow & Gate - r^2=0.34 n=17 P<0.02

Figure D2c



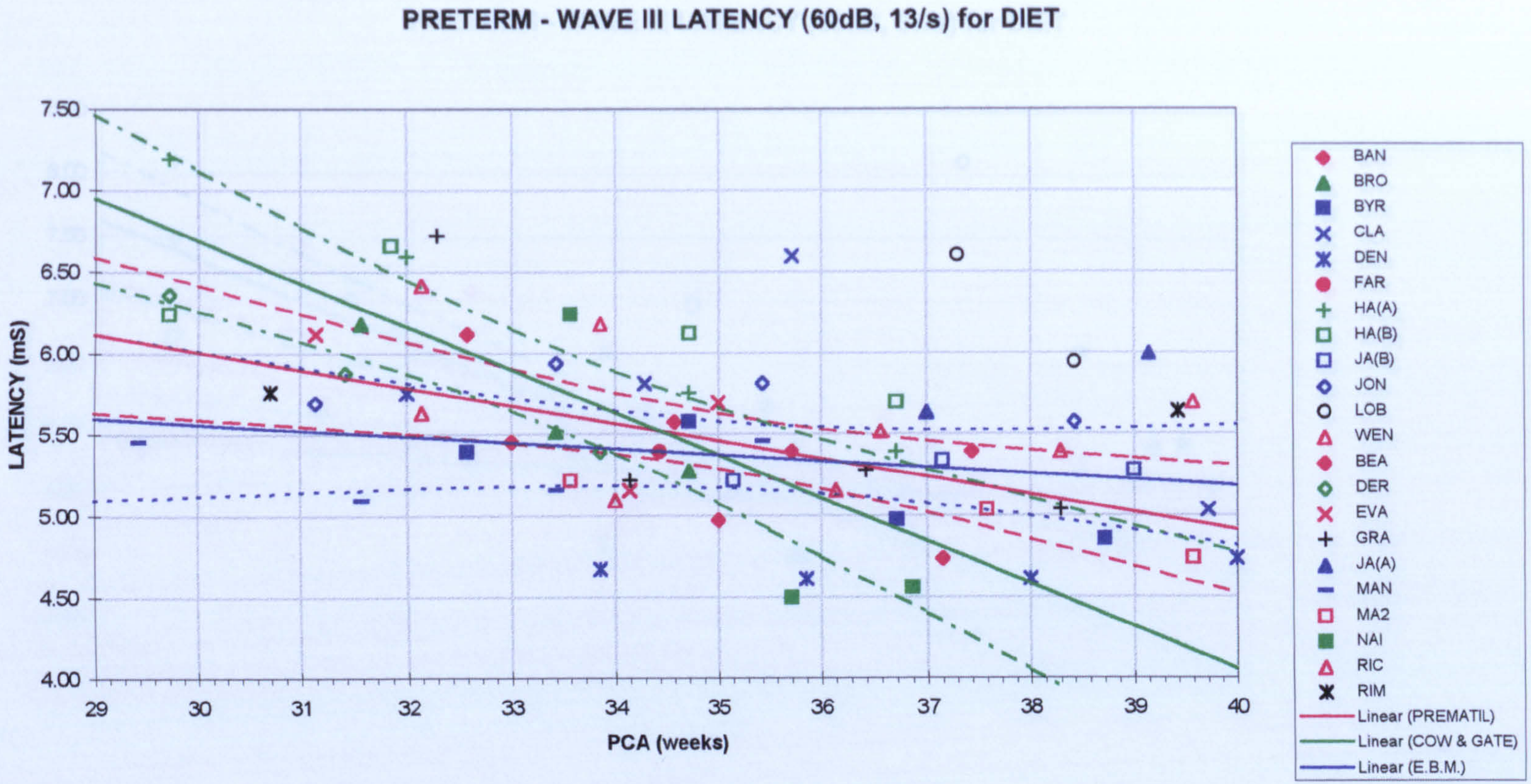
E.B.M. - r^2=0.04 n=25 P>0.05 Prematil - r^2=0.08 n=22 P>0.05 Cow & Gate - r^2=0.25 n=17 P<0.05

Figure D3c



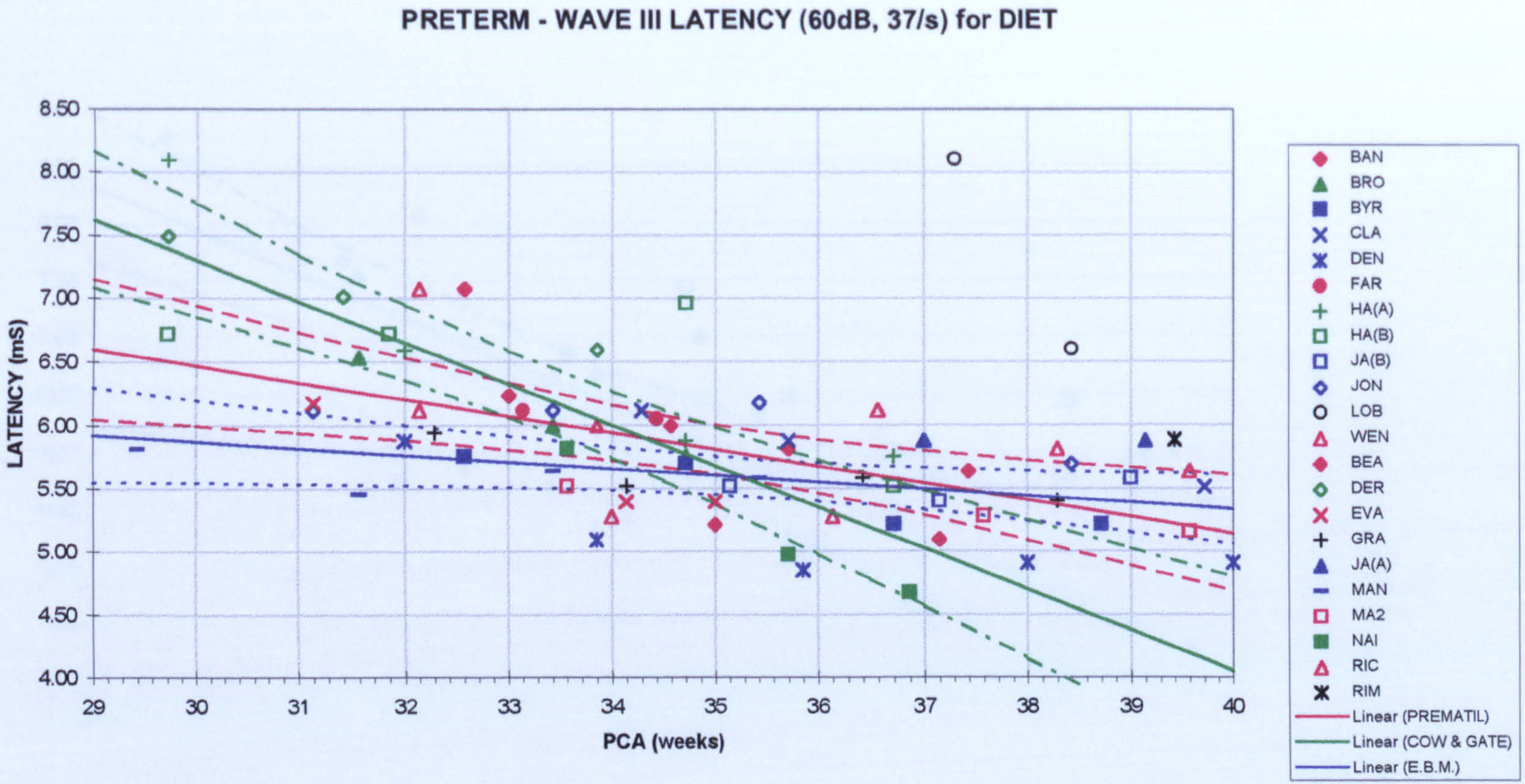
E.B.M. - r^2=0.03 n=26 P>0.05 Prematil - r^2=0.11 n=22 P>0.05 Cow & Gate - r^2=0.55 n=17 P<0.001

Figure D4c



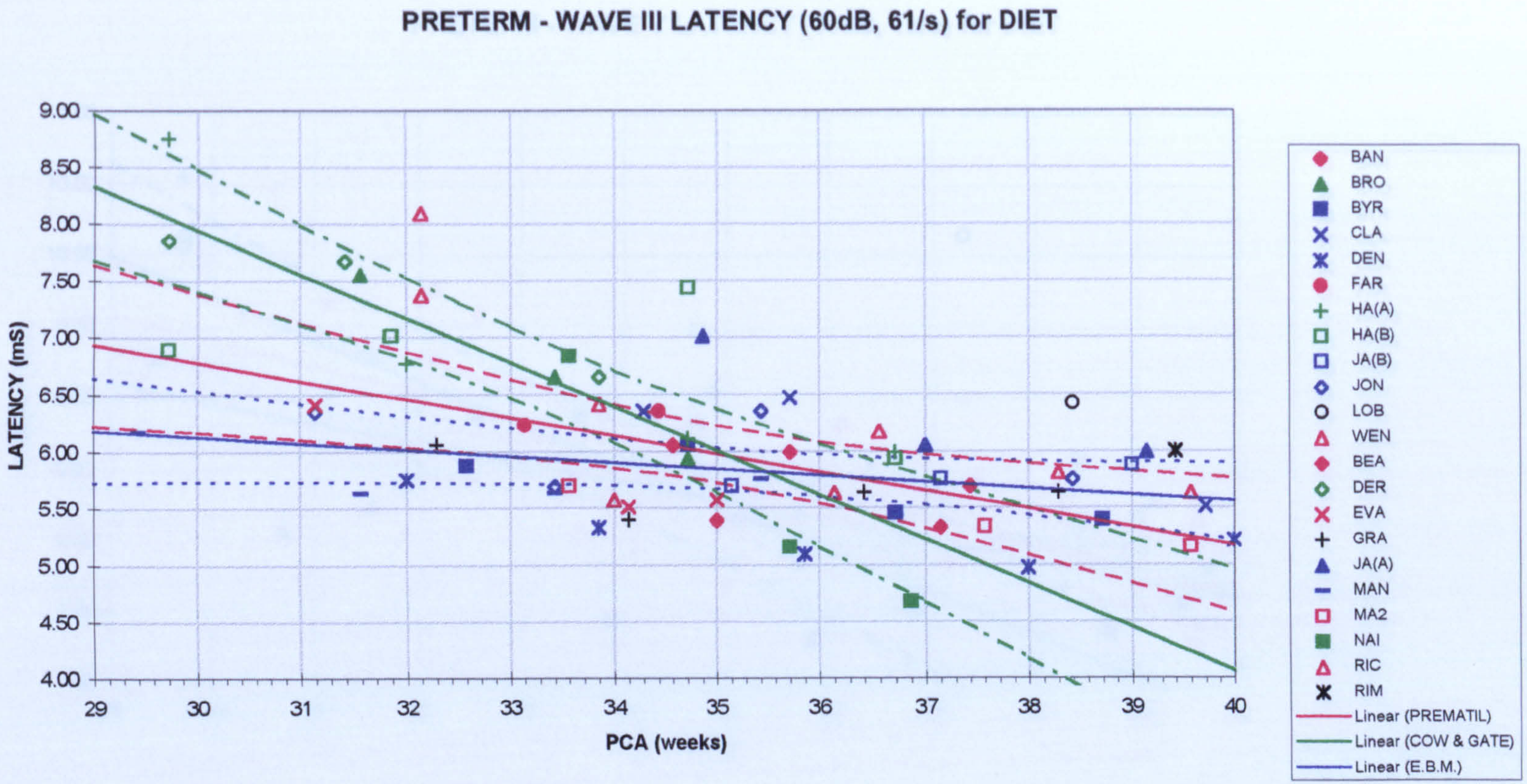
E.B.M. - r^2=0.05 n=25 P>0.05 Prematil - r^2=0.31 n=22 P<0.01 Cow & Gate - r^2=0.58 n=17 P<0.001

Figure D5c



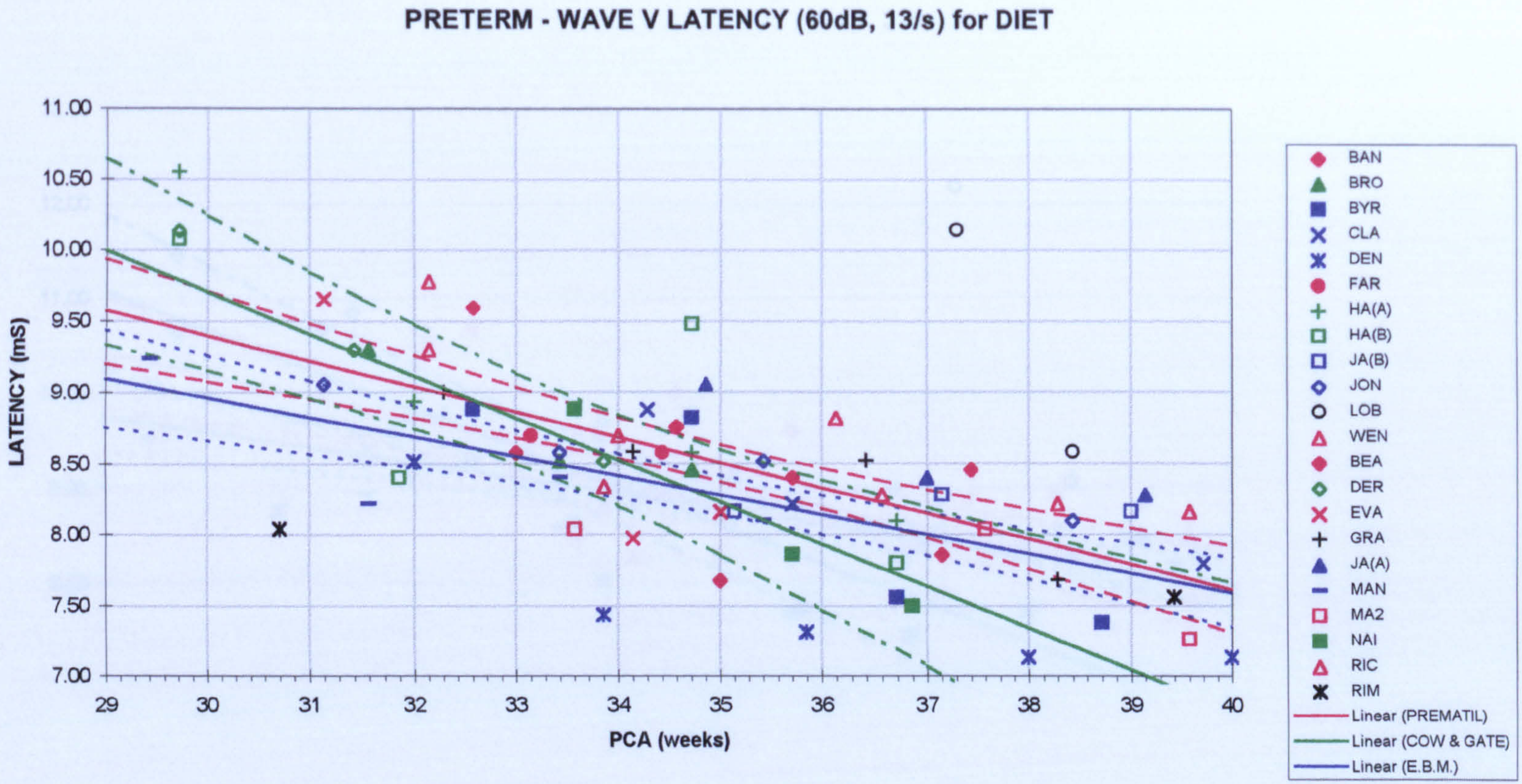
E.B.M. - r^2=0.17 n=25 P<0.05 Prematil - r^2=0.34 n=23 P<0.005 Cow & Gate - r^2=0.70 n=17 P<0.001

Figure D6c



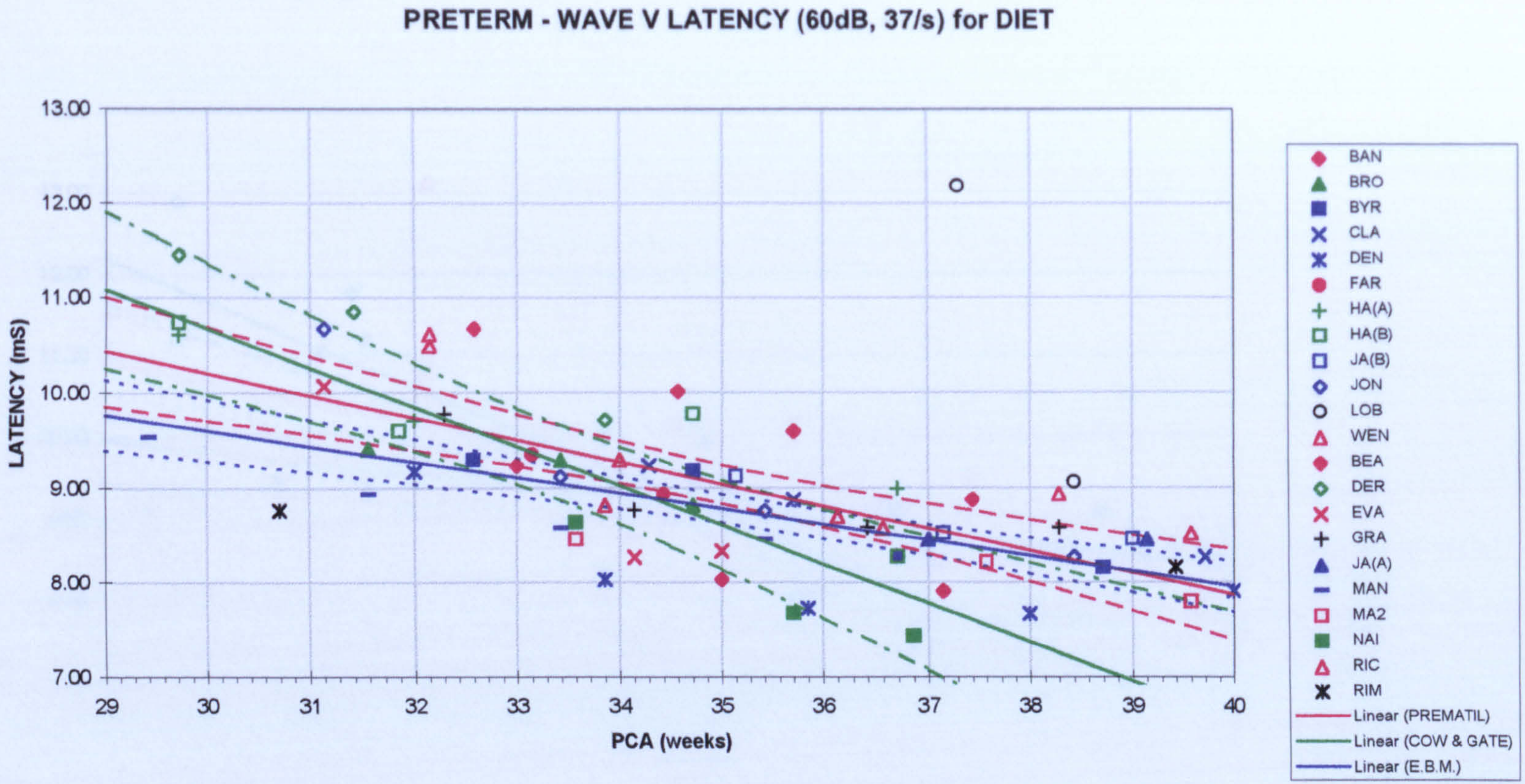
E.B.M. - r^2=0.11 n=26 P>0.05 Prematil - r^2=0.41 n=21 P<0.005 Cow & Gate - r^2=0.68 n=17 P<0.001

Figure D7c



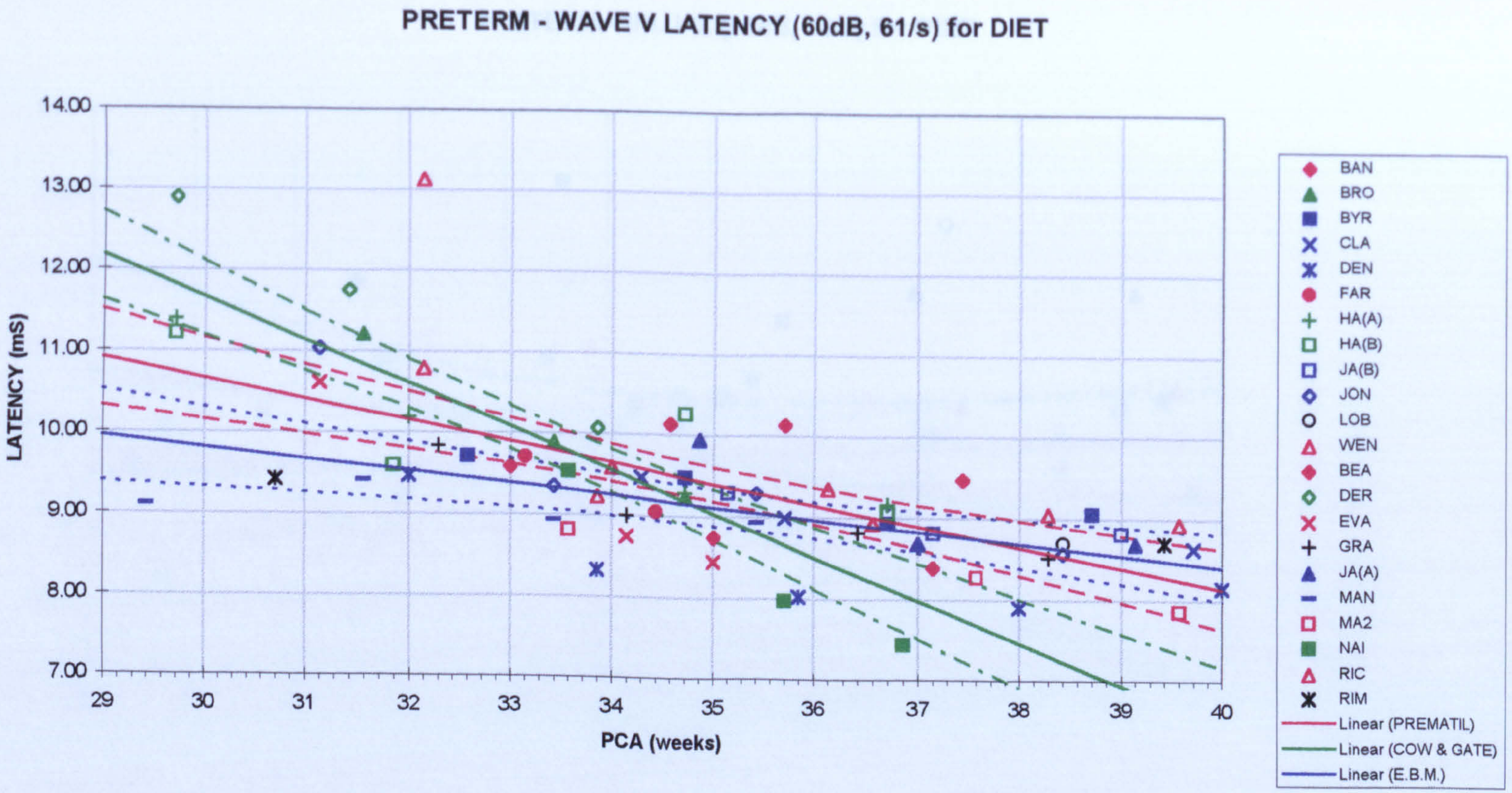
E.B.M. - r^2=0.41 n=26 P<0.001 Prematil - r^2=0.51 n=23 P<0.001 Cow & Gate - r^2=0.76 n=17 P<0.001

Figure D8c



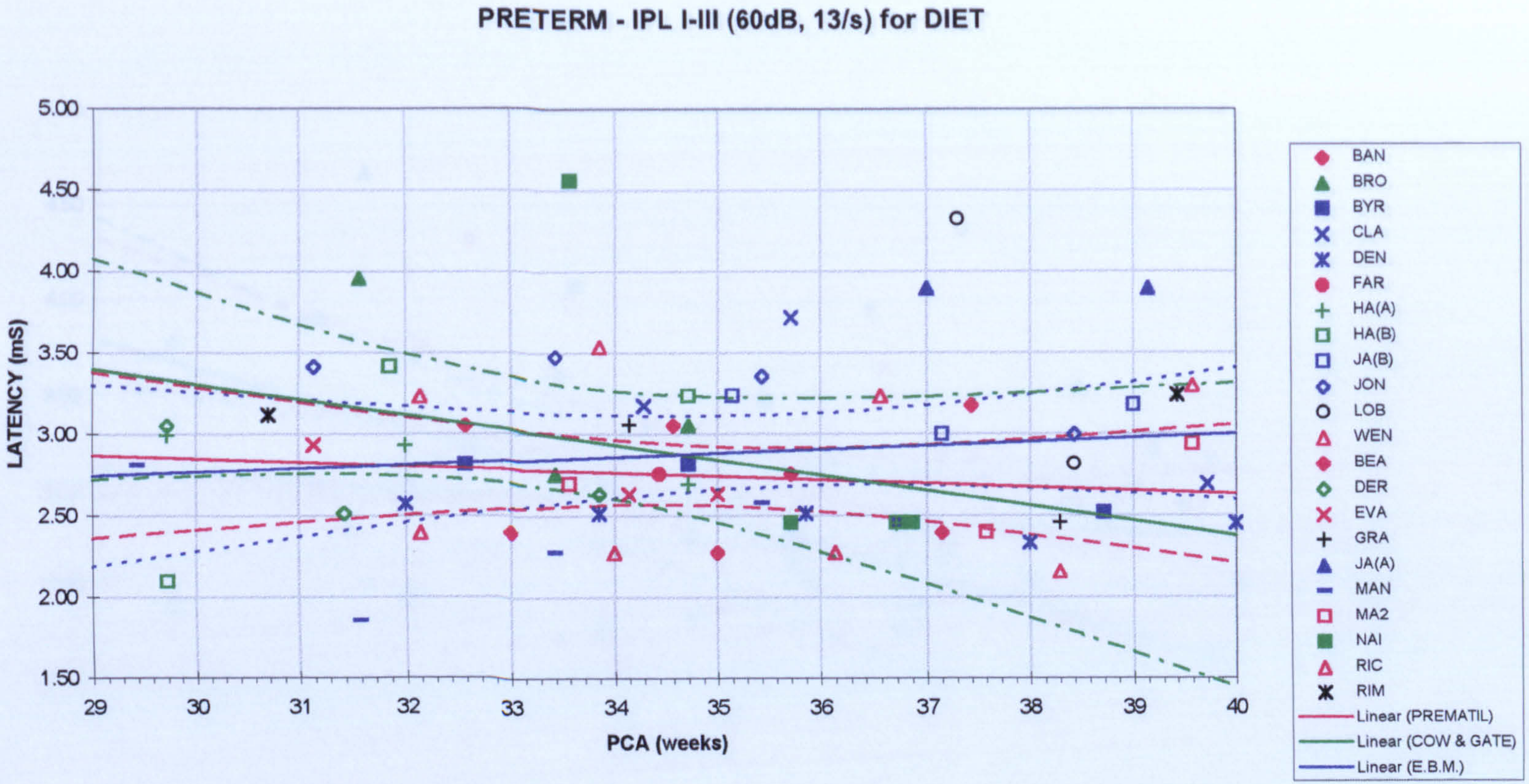
E.B.M. - r^2=0.52 n=25 P<0.001 Prematil - r^2=0.46 n=23 P<0.001 Cow & Gate - r^2=0.74 n=17 P<0.001

Figure D9c



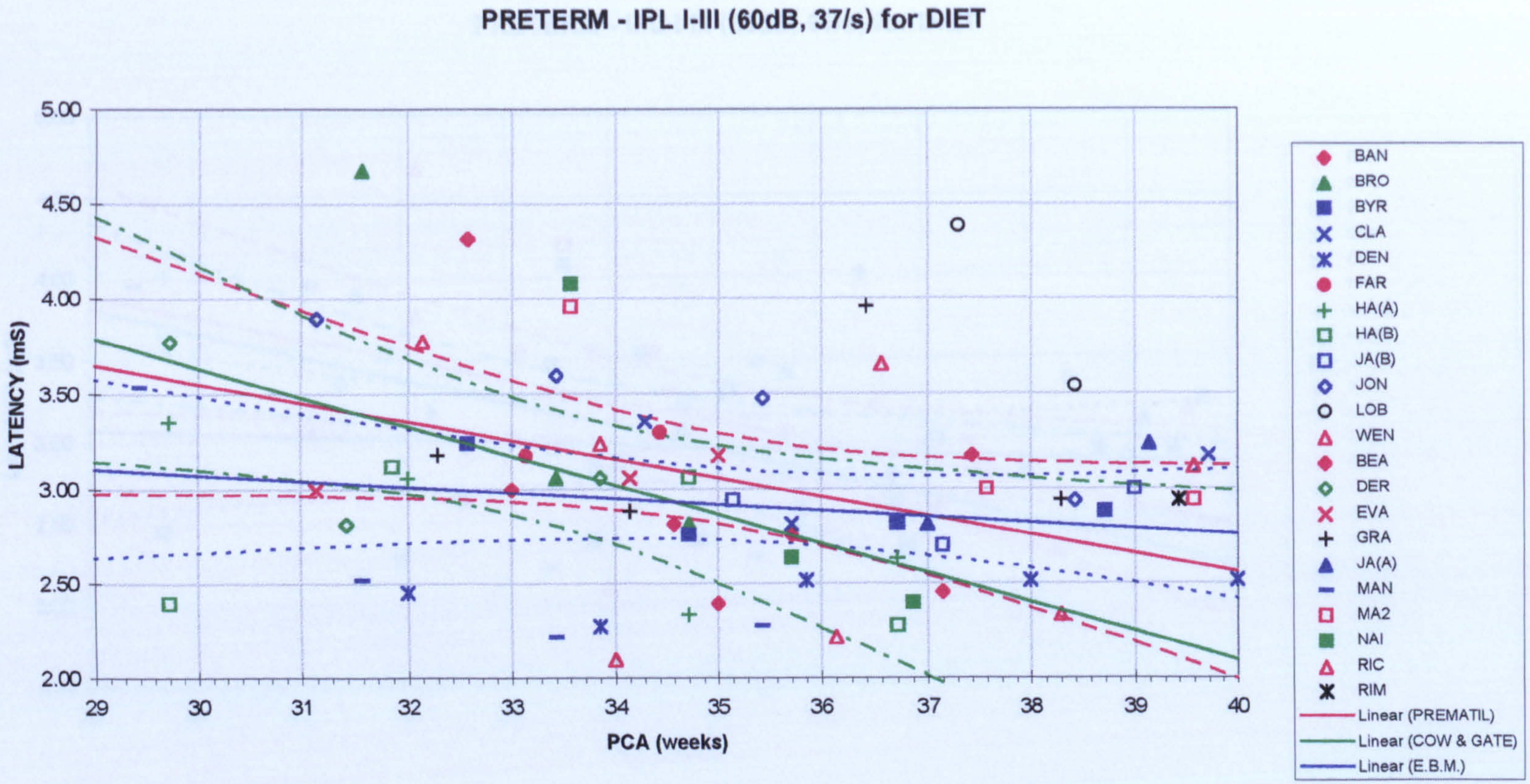
E.B.M. - r^2=0.38 n=26 P<0.001 Prematil - r^2=0.39 n=22 P<0.001 Cow & Gate - r^2=0.74 n=17 P<0.001

Figure D10c



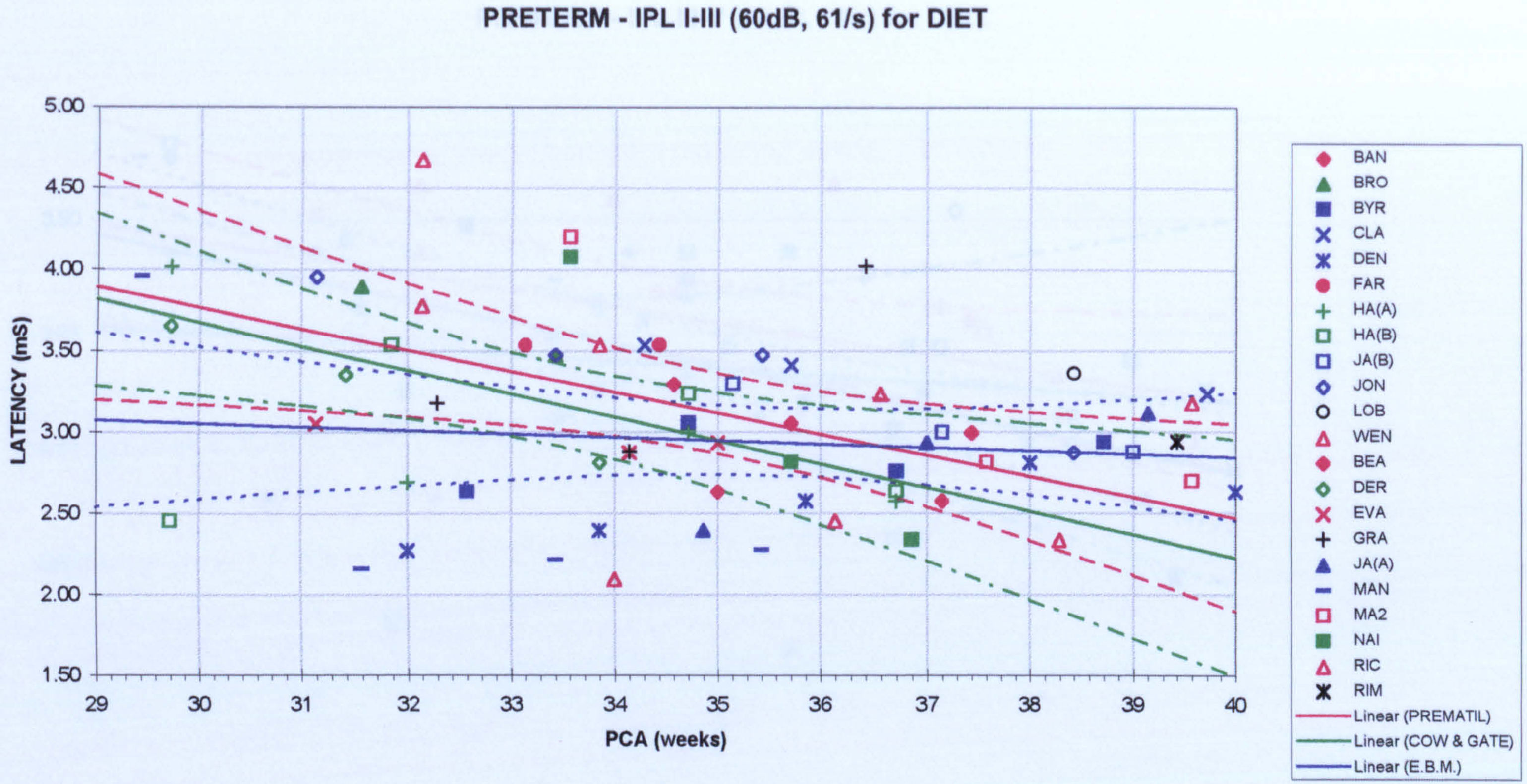
E.B.M. - r^2=0.02 n=25 P>0.05 Prematil - r^2=0.00 n=22 P>0.05 Cow & Gate - r^2=0.04 n=17 P>0.05

Figure D11c



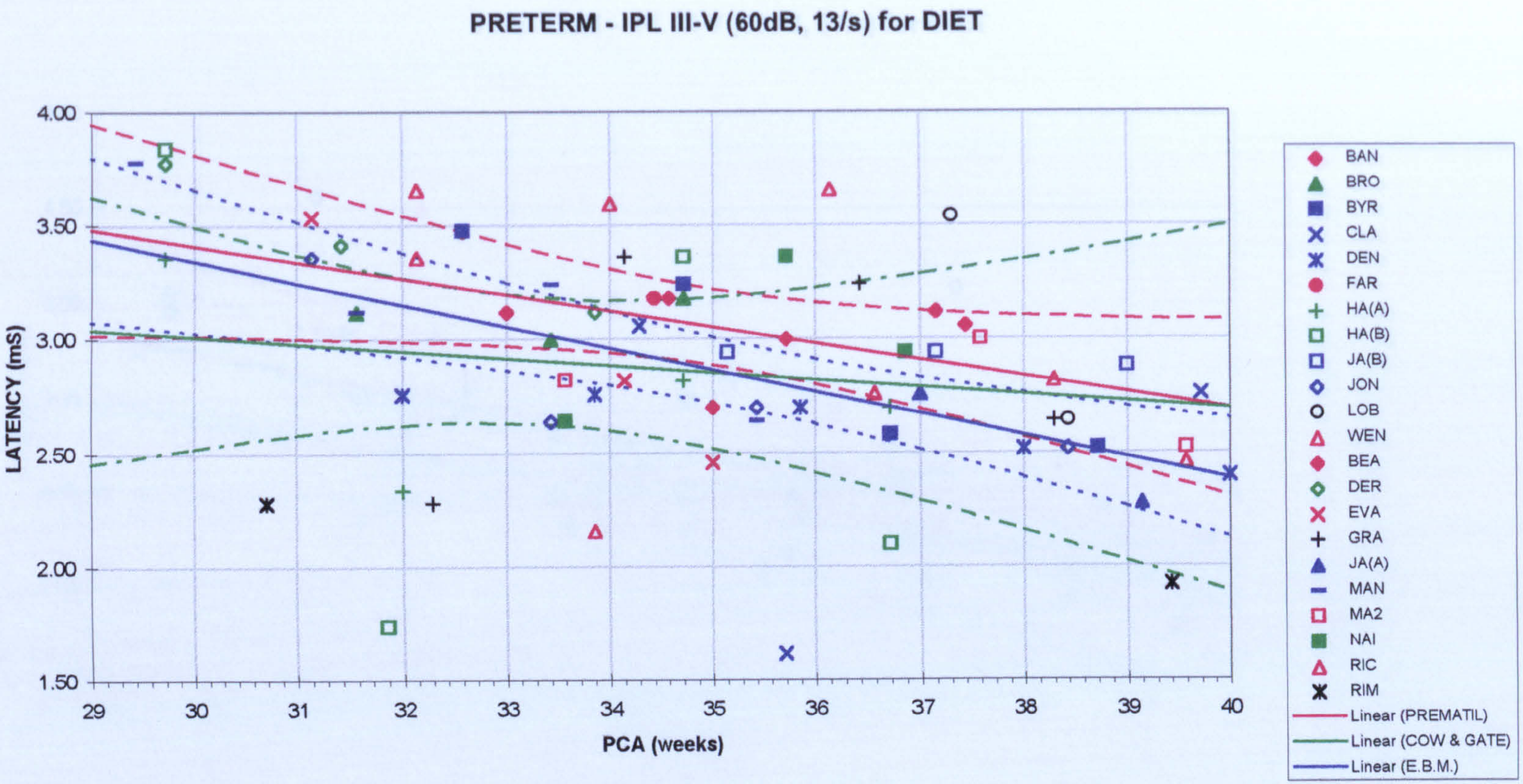
E.B.M. - r^2=0.04 n=25 P>0.05 Prematil - r^2=0.12 n=22 P>0.05 Cow & Gate - r^2=0.23 n=17 P>0.05

Figure D12c



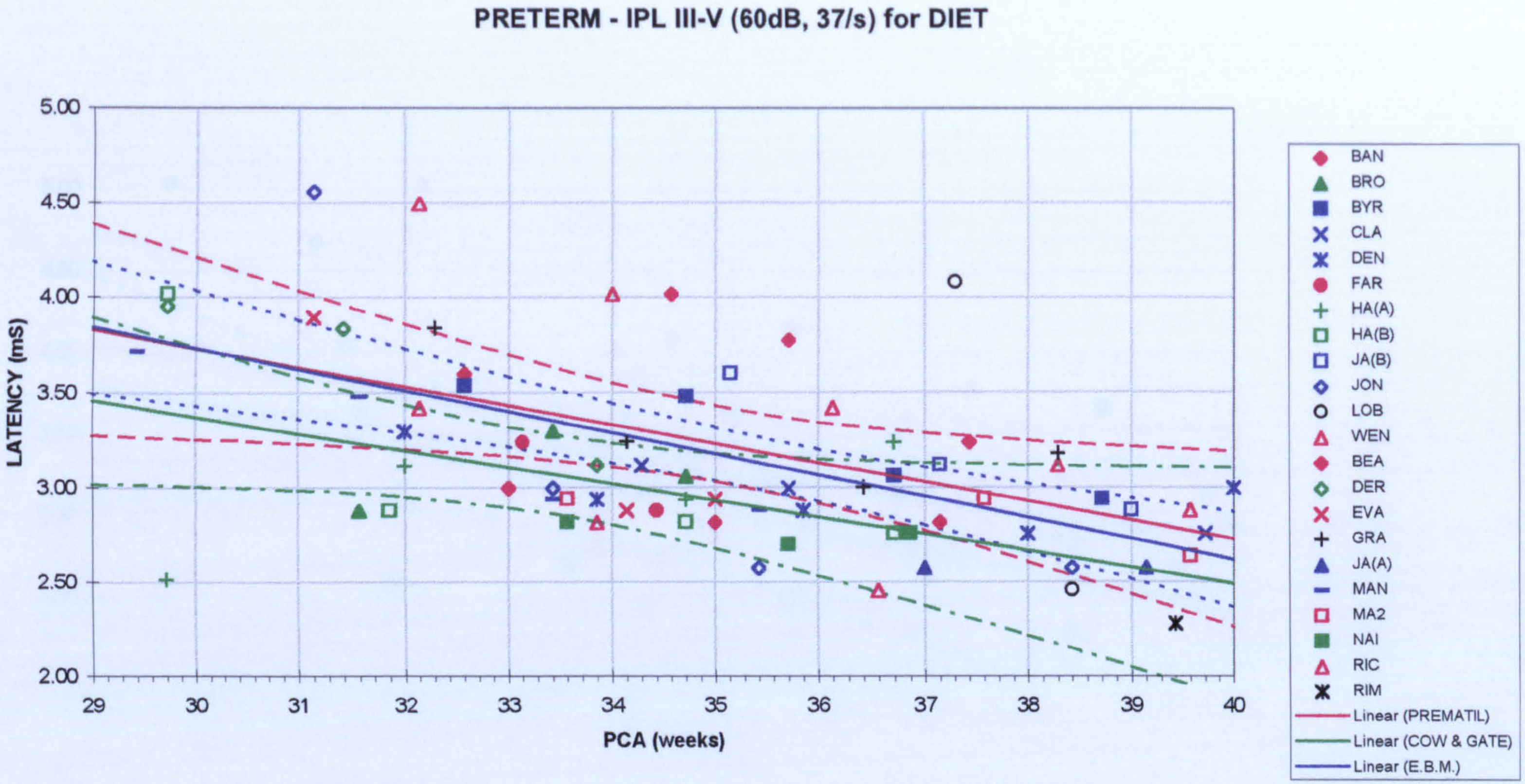
E.B.M. - r^2=0.01 n=26 P>0.05 Prematil - r^2=0.27 n=21 P<0.02 Cow & Gate - r^2=0.28 n=17 P<0.05

Figure D13c



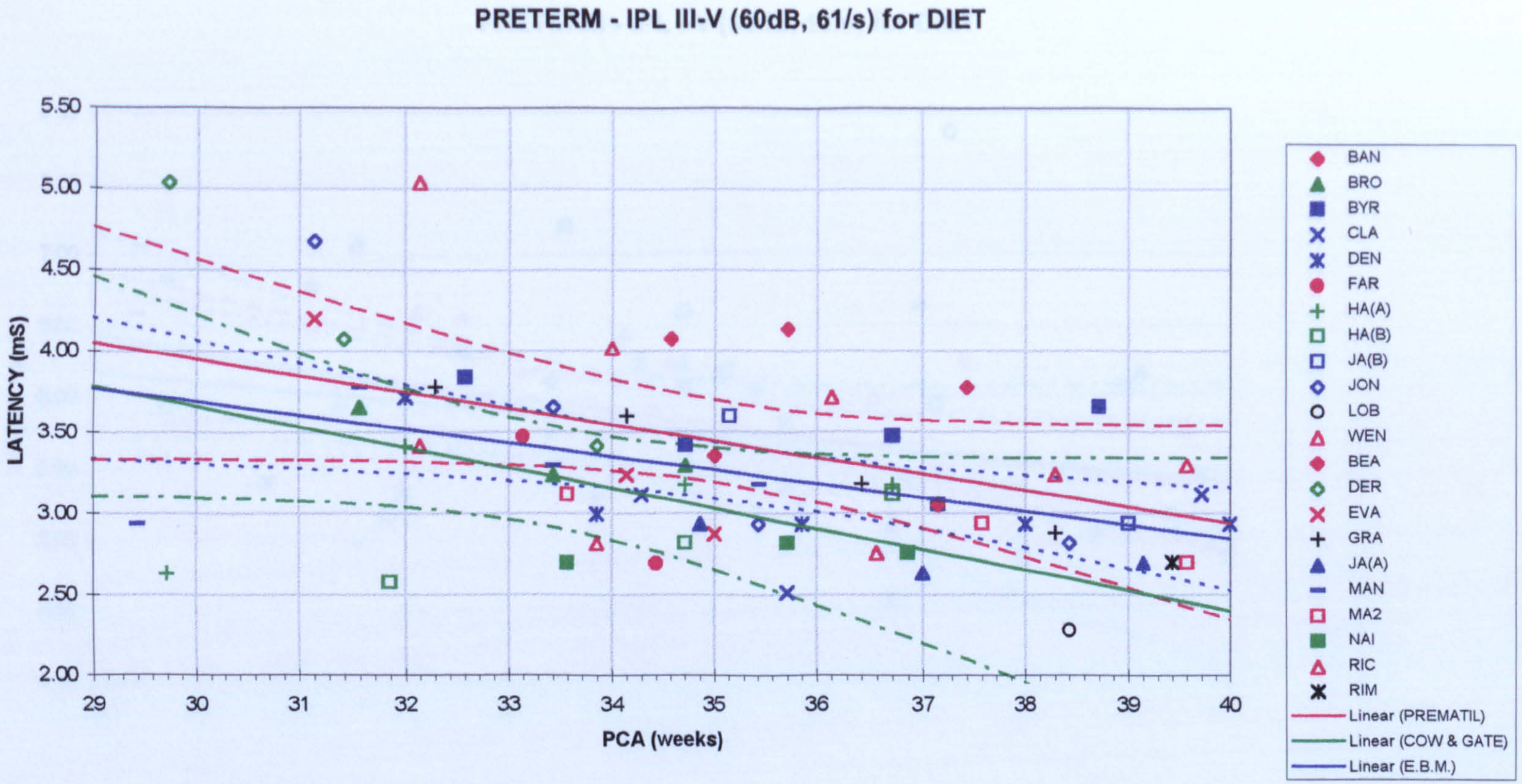
E.B.M. - r^2=0.40 n=25 P<0.001 Prematil - r^2=0.24 n=22 P<0.025 Cow & Gate - r^2=0.14 n=17 P>0.05

Figure D14c



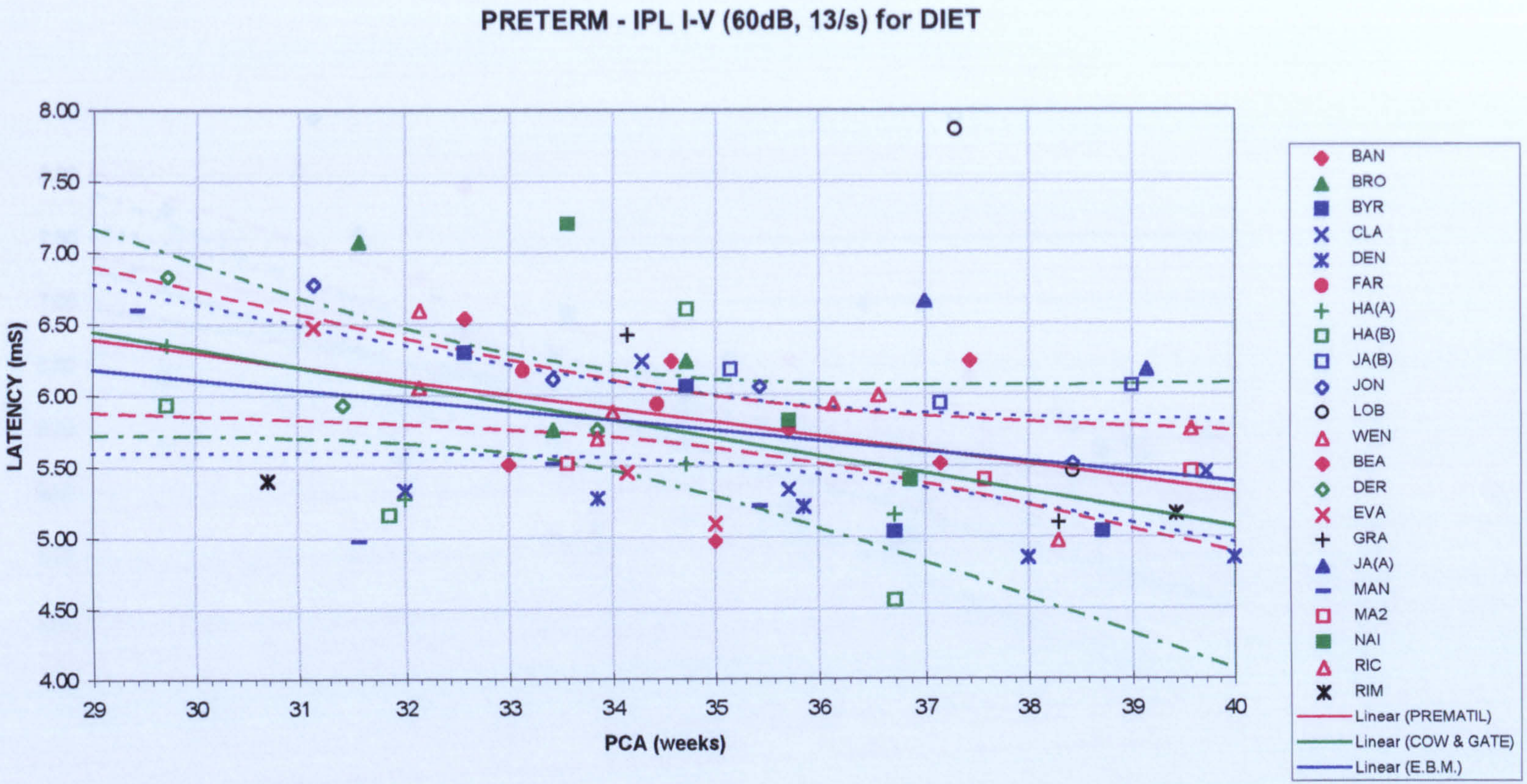
E.B.M. - r^2=0.48 n=25 P<0.001 Prematil - r^2=0.26 n=23 P<0.02 Cow & Gate - r^2=0.24 n=17 P<0.05

Figure D15c



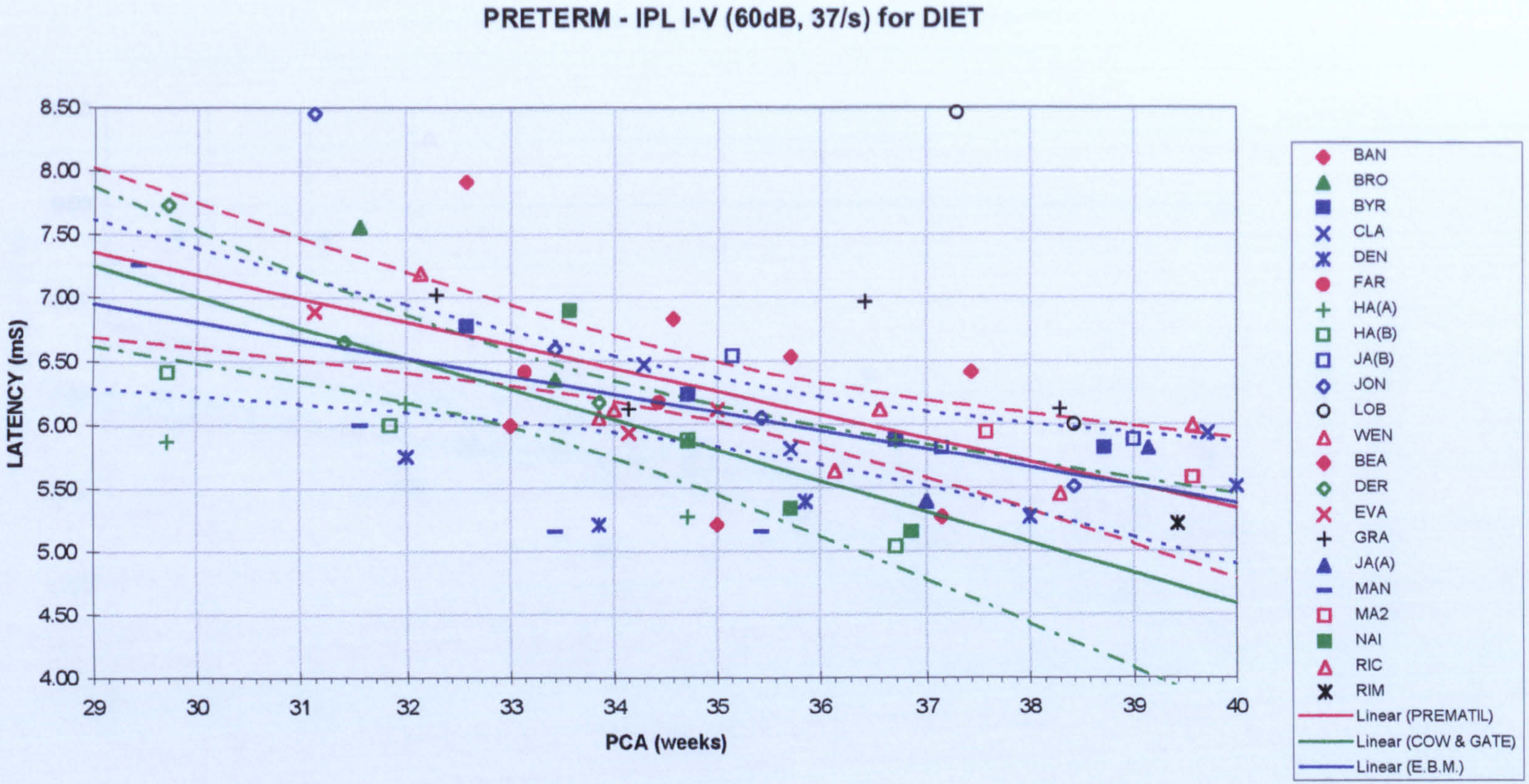
E.B.M. - r^2=0.26 n=26 P<0.01 Prematil - r^2=0.19 n=21 P<0.05 Cow & Gate - r^2=0.20 n=16 P>0.05

Figure D16c



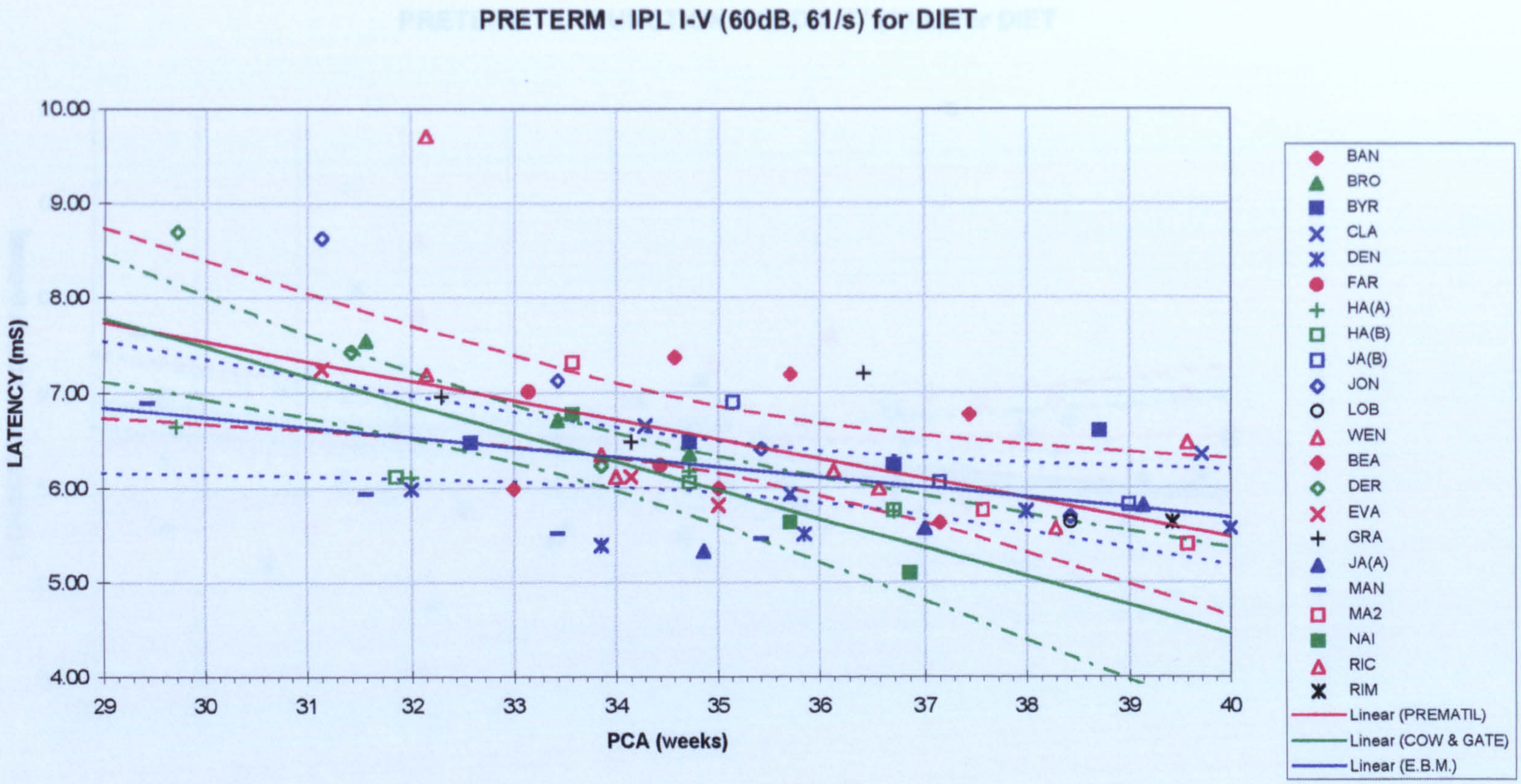
E.B.M. - r^2=0.12 n=25 P>0.05 Prematil - r^2=0.23 n=23 P<0.025 Cow & Gate - r^2=0.21 n=17 P>0.05

Figure D17c



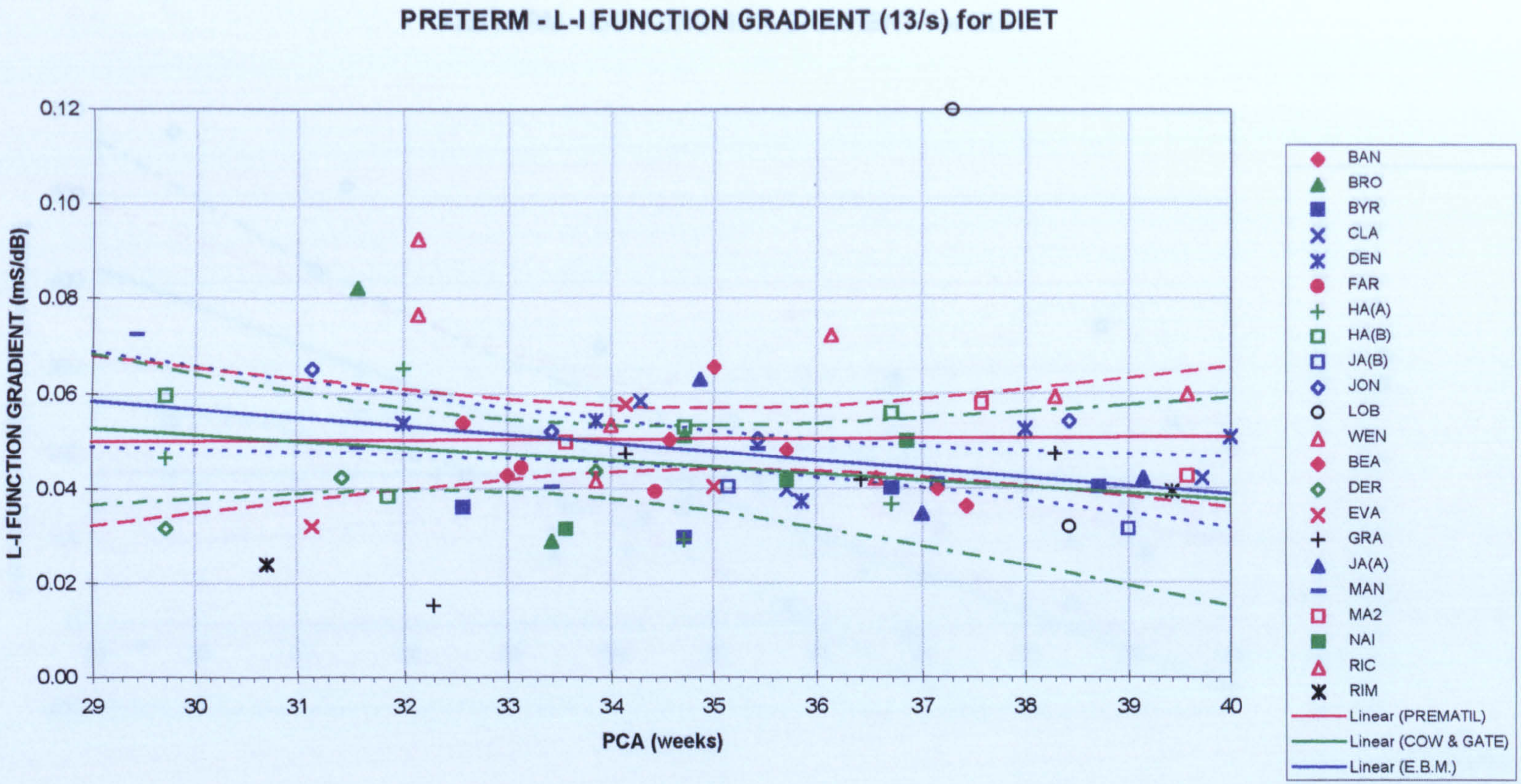
E.B.M. - r^2=0.29 n=25 P<0.01 Prematil - r^2=0.37 n=22 P<0.005 Cow & Gate - r^2=0.48 n=17 P<0.005

Figure D18c



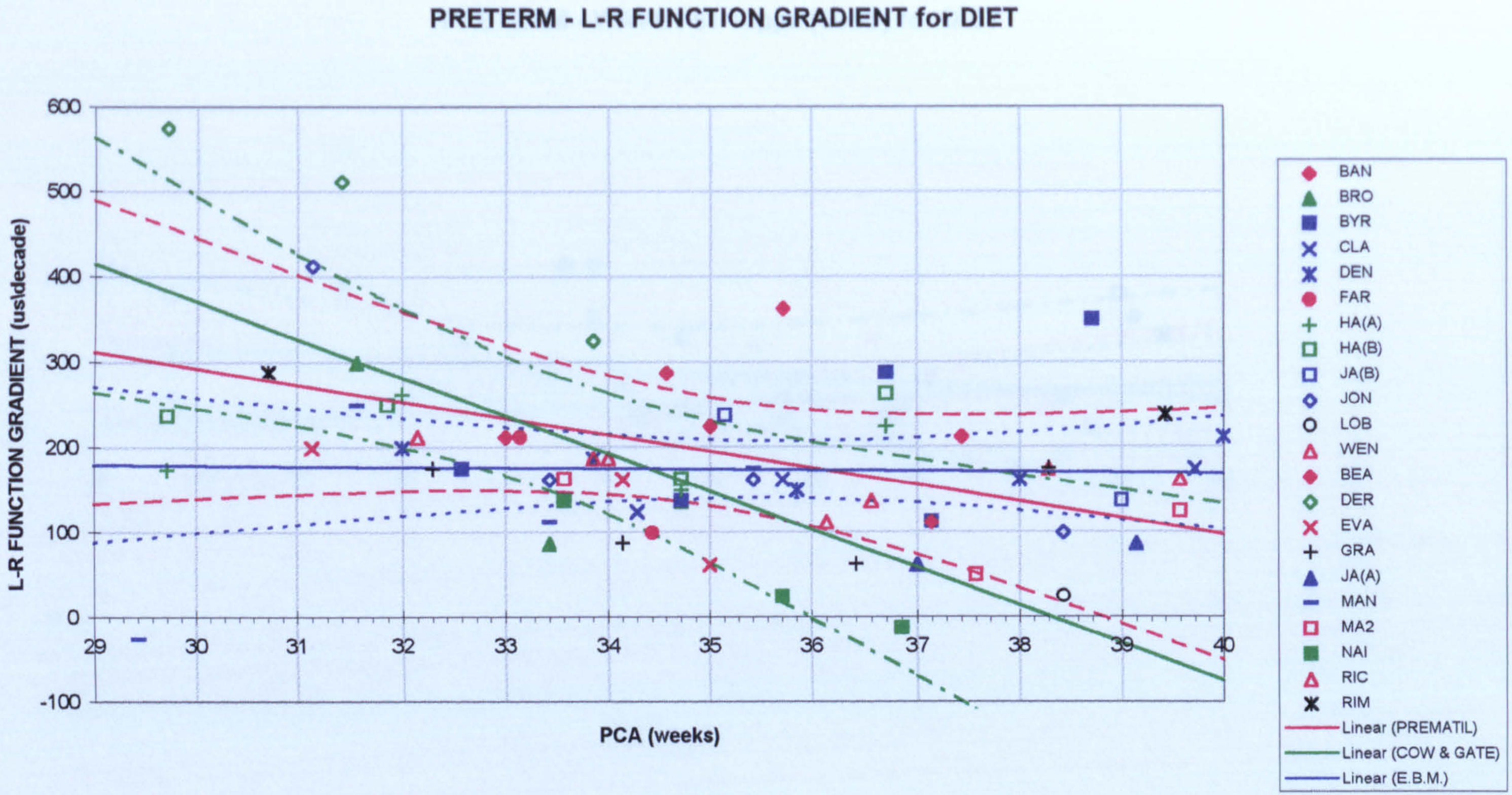
E.B.M. - r^2=0.17 n=26 P<0.05 Prematil - r^2=0.32 n=22 P<0.01 Cow & Gate - r^2=0.62 n=16 P<0.001

Figure D19c



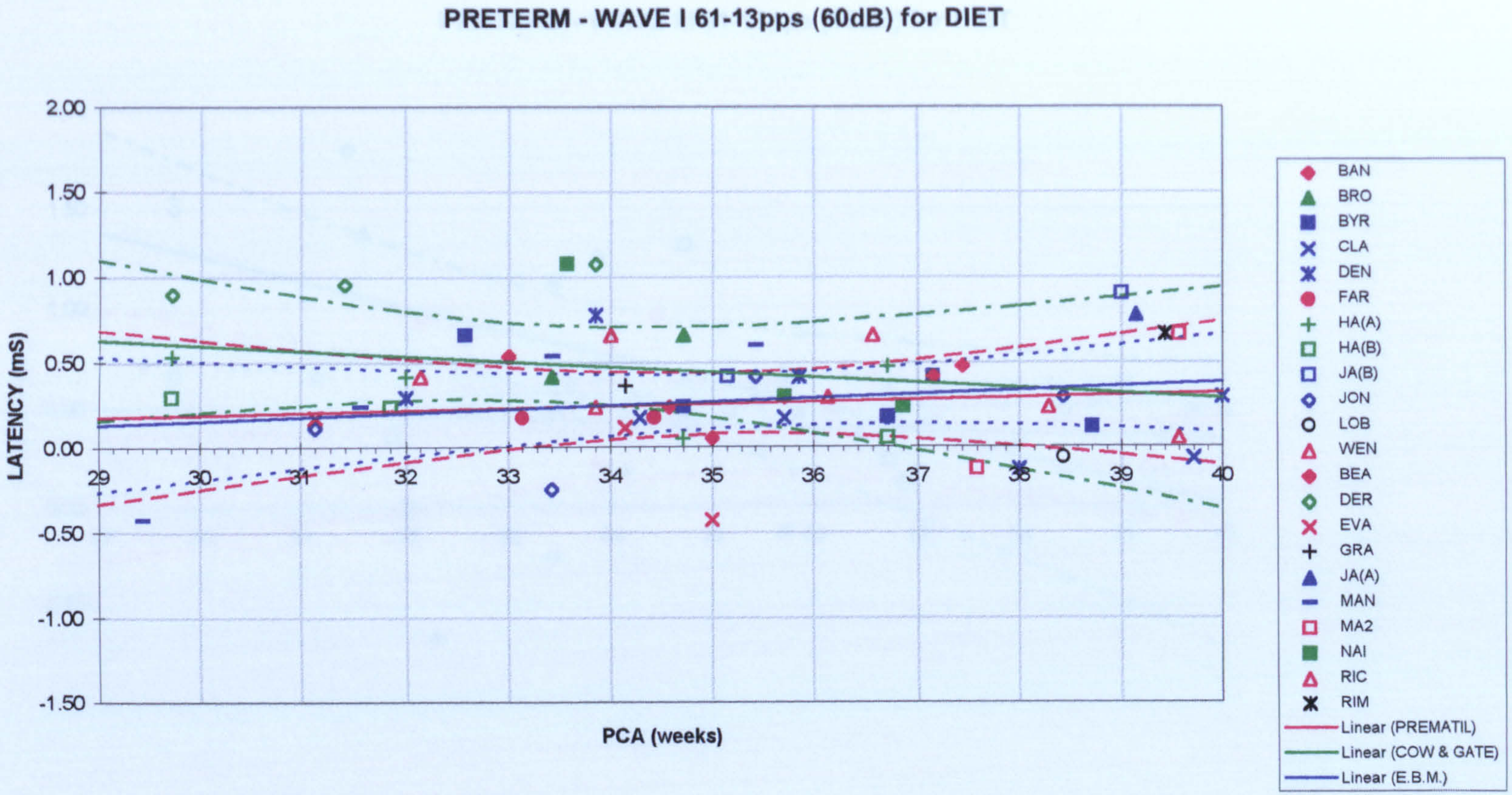
E.B.M. - r^2=0.22 n=26 P<0.02 Prematil - r^2=0.01 n=23 P>0.05 Cow & Gate - r^2=0.02 n=17 P>0.05

Figure D20c



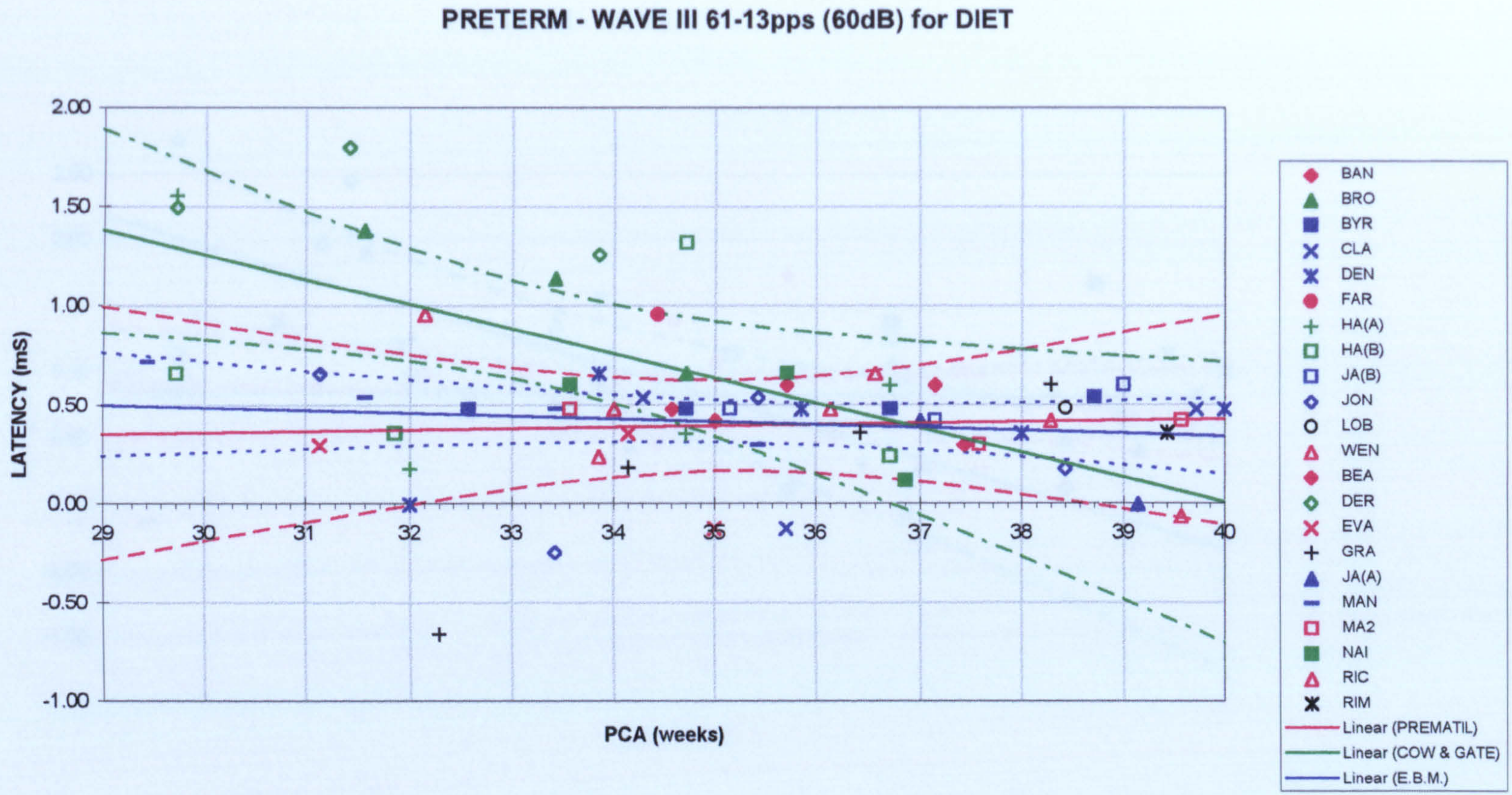
E.B.M. - r^2=0.08 n=24 P>0.05 Prematil - r^2=0.15 n=22 P>0.05 Cow & Gate - r^2=0.24 n=16 P>0.05

Figure D22c



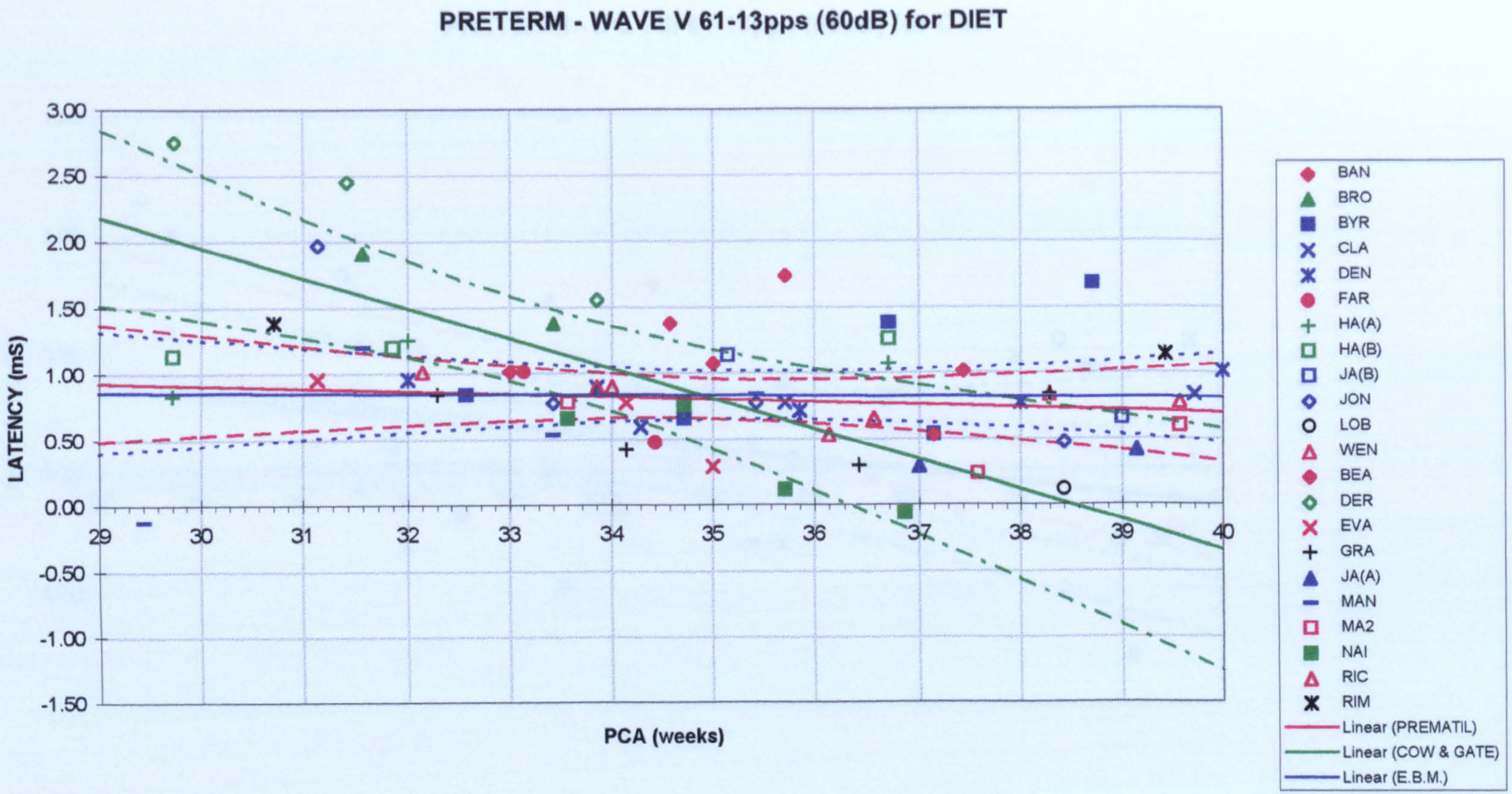
E.B.M. - r^2=0.06 n=25 P>0.05 Prematil - r^2=0.03 n=21 P>0.05 Cow & Gate - r^2=0.08 n=17 P>0.05

Figure D24c



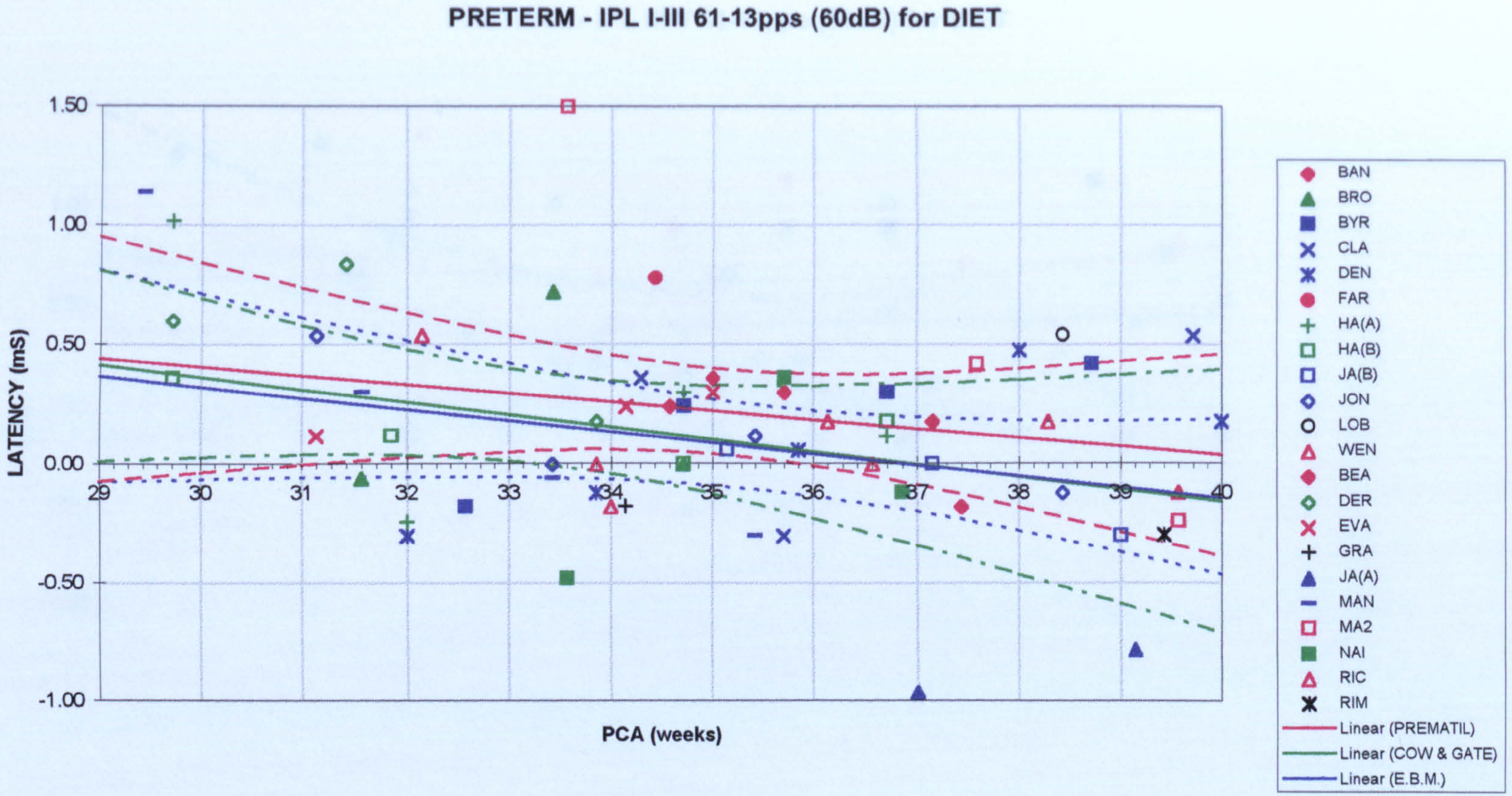
E.B.M. - r^2=0.02 n=25 P>0.05 Prematil - r^2=0.17 n=20 P>0.05 Cow & Gate - r^2=0.29 n=17 P<0.05

Figure D26c



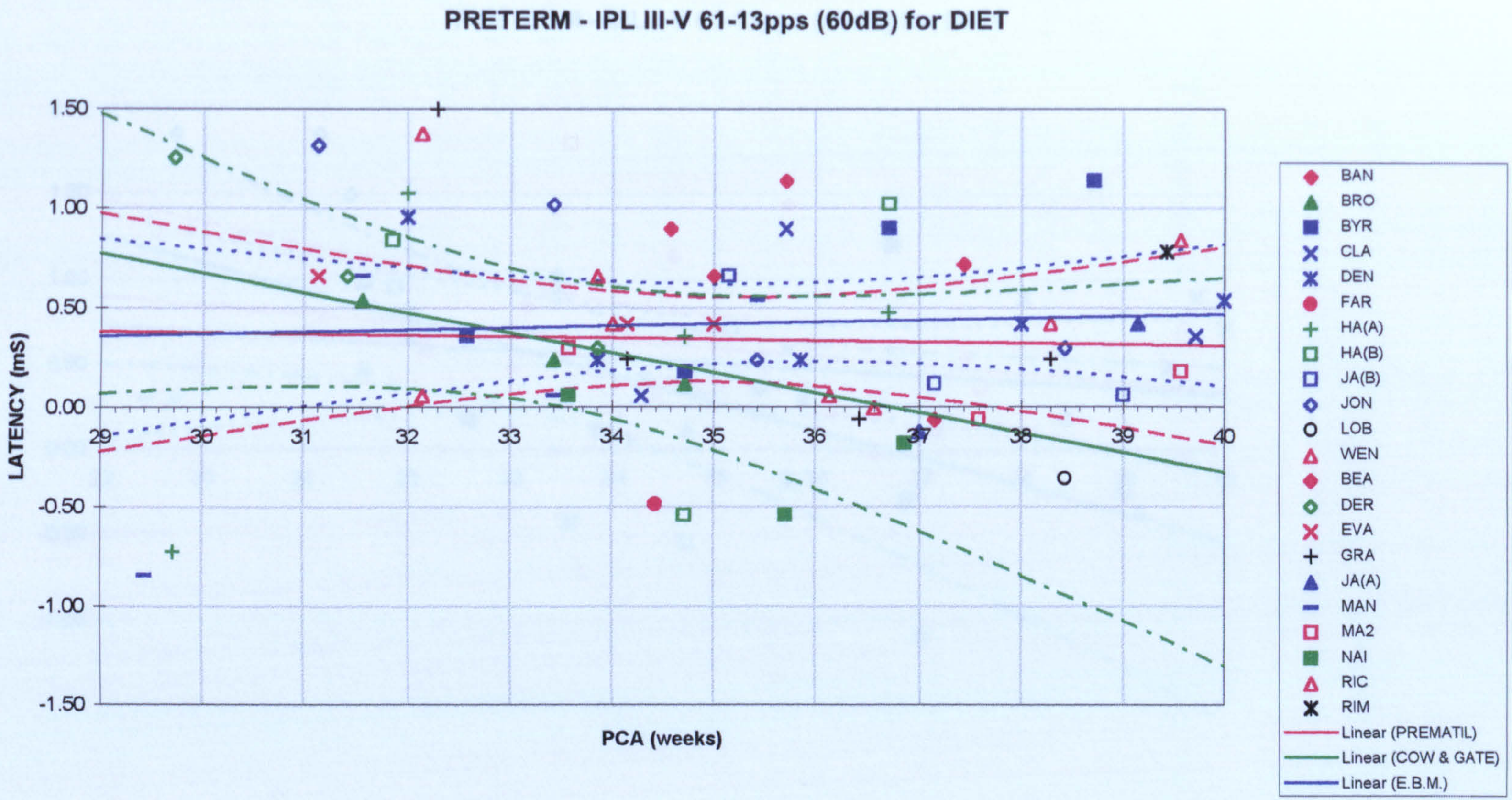
E.B.M. - r^2=0.00 n=25 P>0.05 Prematil - r^2=0.09 n=21 P>0.05 Cow & Gate - r^2=0.34 n=17 P<0.02

Figure D28c



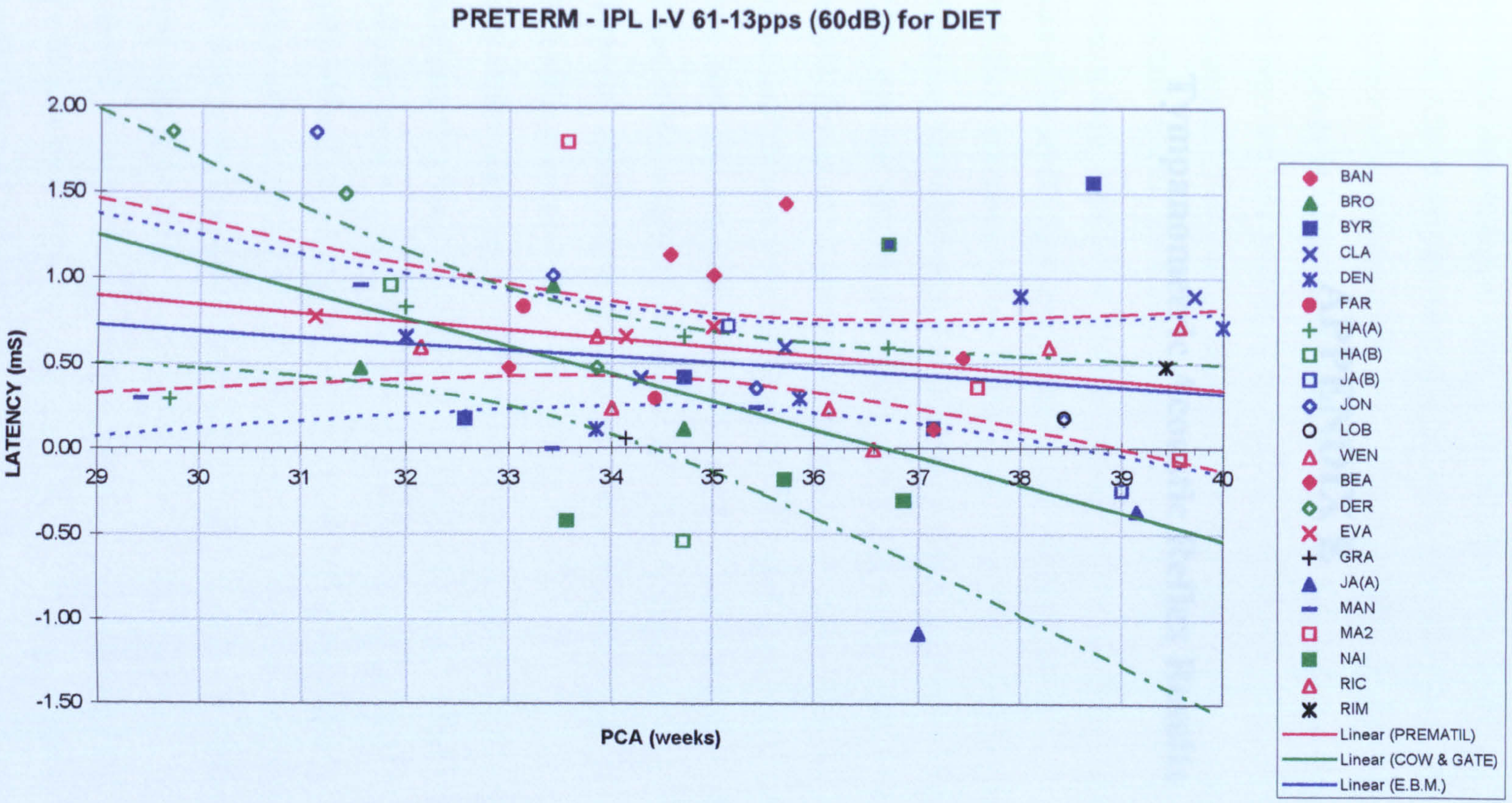
E.B.M. - r^2=0.09 n=25 P>0.05 Prematil - r^2=0.18 n=19 P>0.05 Cow & Gate - r^2=0.19 n=17 P>0.05

Figure D30c



E.B.M. - r^2=0.00 n=25 P>0.05 Prematil - r^2=0.03 n=20 P>0.05 Cow & Gate - r^2=0.05 n=16 P>0.05

Figure D32c



E.B.M. - r^2=0.03 n=25 P>0.05 Prematil - r^2=0.13 n=21 P>0.05 Cow & Gate - r^2=0.02 n=16 P>0.05

APPENDIX E

Tympanometric Acoustic Reflex Results

The Acoustic Reflex (AR) assessment undertaken during this work was concerned with the acoustic reflex threshold (ART). Due to time restraints, it was not possible to obtain morphological data of immittance changes during the reflex. The ability to detect ARs in the newborn population is highly dependent on the probe tone frequency implemented. It is generally reported that an 800Hz probe tone will allow for elicitation of reflexes in infants at the same rate as for the adult population. Weatherby and Bennett[1] reported that 1400Hz is the optimum probe tone frequency. The neonate tympanic membrane (TM), when stimulated at low frequency, presents a reduced impedance which 'shunts' the higher impedance of the middle ear (ME). This situation, of measuring a high impedance system through a low impedance medium, makes identification of the small impedance changes of the AR impossible.

The GSI33 instrument allows testing with 226, 678 and 1000Hz probe tones. A 1000Hz frequency was implemented in order to achieve optimum elicitation. Activator signals of 226, 678 and 1000Hz pure tones were used to detect ARs, response to broad band noise (BBN) was also examined. A 5dB activator step was employed with tests being repeated until reflexes were confirmed over the presence of behavioural responses. This process was time consuming with the more restless infants. It should first be mentioned that, whilst a high percentage of term infants displayed ARs for the range of available activator stimuli, there were no ARs elicited from any of the seven preterm infants tested with tympanometry.

Many studies during the 1970's reported the low incidence of ARs in neonates and young infants, they found very low elicitation levels. Allred[2] found 85% of neonates to have reflexes present with a 660Hz probe tone. Margolis and Popelka[3] elicited reflexes in 69-94% of infants depending upon the activator signal (500-4000Hz, BBN). Testing with a BBN activator, Weatherby and Bennett[1] reported 100% reflex elicitation in neonates with probe frequencies between 800 and 1800Hz. At 1200Hz probe tone, they reported reflexes for at least one activator in 25 of 28 neonates.

It should be remembered that the majority of early reports on the AR are concerned with elicitation of the contralateral reflex. This was originally necessary due to the problematic nature of ipsilateral recordings which necessitate the introduction of both probe tone and activating signals to a single ear. It was initially easier to present the activating signal to the opposite ear, relying on the cross over of nerves in the brainstem.

For this study, only the ipsilateral AR was assessed due to the difficulty found when trying to keep both ipsilateral and contralateral probe tips in place.

Testing was implemented with a 1000Hz probe tone and activators of 500Hz, 1000Hz, 4000Hz and BBN. It was found that 93% (25 of 27 infants) of the term infants presented an ipsilateral reflex with one of the mentioned activating signals. One infant was too restless to test for ARs. Elicitation for particular activators; 500Hz, 1000Hz, 4000Hz and BBN were 63, 82, 59 and 89% respectively. There is clearly reduced elicitation for 500 and 4000Hz activators. Of the reflexes elicited, the mean dB level was found to be 94 ($P_{0.05}$=4.4), 91 ($P_{0.05}$=2.1) and 89dB ($P_{0.05}$=2.7) for 500, 1000 and 4000Hz activators respectively. This reduction in activator level with increasing frequency is seen with the majority of individual data. The mean for BBN was found to be 65dB ($P_{0.05}$=4.1).

Gelfand[4] suggested that it is wise to consider reasons other than anatomical and physiological maturation for the ART differences between adults and infants. This is due to the comparability of adult and infant ARTs, and the fact that the neonate auditory system is sufficiently mature for elicitation of the AR. It has already been mentioned that elevation or absence of ARs at low frequency probe tones is due to the resonant characteristics of the infant ME. Gelfand[4] pointed out two other significant factors; namely, transient conductive conditions and behavioural responses. A conductive condition could have the effect of lessening activator signal levels reaching the cochlea, or could decrease the effectiveness of monitoring immittance changes caused by a reflex. Behavioural responses were reported as a problem in neonatal AR testing by Keith[5]. Bennett[6] reported motor response thresholds of 90dB for 1000Hz probe frequency and 75dB for BBN, these levels are within the ART ranges found with this data.

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APPENDIX F

Tympanic Membrane Finite Element Model

A finite element model of the infant tympanic membrane (TM) was constructed using PAFEC Version 8.5 software. The model was designed to investigate the particularly low resonant frequency characteristics that were found during tympanometric testing. The effect of TM properties (in relation to the rest of the middle ear (ME) system), and how this relates to the overall mechanical characteristics were to be examined.

There have been many studies on the mechanical properties of body (human and animal) structures and tissues. However, information on the complex mechanical properties of the neonate TM (in particular the premature TM) is currently limited. A number of useful references and sources of information from previous modelling studies and experiments on tissue properties are included at the end of this appendix.

Time was not sufficient to continue with this area of research. However, this model may be useful for further work in this field.

MODEL 5MM DIAM. INFANT
C
NODES
NODE.NUMBER AXIS.NUMBER X Y Z
1 1 0 0.003125 0.0125
2 1 0.001563 0.003125 0.0125
3 1 0.003125 0.003125 0.0125
4 1 0.003125 0.001563 0.0125
5 1 0.003125 0 0.0125
6 1 0.003125 -0.001563 0.0125
7 1 0.003125 -0.003125 0.0125
8 1 0.001563 -0.003125 0.0125
9 1 0 -0.003125 0.0125
10 1 -.001563 -0.003125 0.0125
11 1 -0.003125 -0.003125 0.0125
12 1 -0.003125 -0.001563 0.0125
13 1 -0.003125 0 0.0125
14 1 -0.003125 0.001563 0.0125
15 1 -0.003125 0.003125 0.0125
16 1 -.001563 0.003125 0.0125
17 3 0.025 90
18 3 0.025 67.5
19 3 0.025 45
20 3 0.025 22.5
21 3 0.025 0
22 3 0.025 337.5
23 3 0.025 315
24 3 0.025 292.5
25 3 0.025 270
26 3 0.025 247.5
27 3 0.025 225
28 3 0.025 202.5
29 3 0.025 180
30 3 0.025 157.5
31 3 0.025 135
32 3 0.025 112.5
33 4 0.014 90 0.004375
34 4 0.0147 45 0.004375
35 4 0.014 0 0.004375
36 4 0.0147 315 0.004375
37 4 0.014 270 0.004375
38 4 0.0147 225 0.004375
39 4 0.014 180 0.004375
40 4 0.0147 135 0.004375
41 1 0 0 0.0125
42 4 0.0142 67.5 0.004375
43 4 0.0142 22.5 0.004375
44 4 0.0142 337.5 0.004375
45 4 0.0142 292.5 0.004375
46 4 0.0142 247.5 0.004375
47 4 0.0142 202.5 0.004375
48 4 0.0142 157.5 0.004375
49 4 0.0142 112.5 0.004375
50 3 0.025 78.75
51 3 0.025 56.25
52 3 0.025 33.75
53 3 0.025 11.25
54 3 0.025 348.75
55 3 0.025 326.25
56 3 0.025 303.75
57 3 0.025 281.25
58 3 0.025 258.75
59 3 0.025 236.25
60 3 0.025 213.75
61 3 0.025 191.25
62 3 0.025 168.75
63 3 0.025 146.25
64 3 0.025 123.75
65 3 0.025 101.25

```
PAFBLOCKS
BLOCK.NUMBER TYPE ELEMENT.TYPE PROPERTIES N1 N2 TOPOLOGY
1 1 43210 1  1 2  1 2 17 18 0 33 42 50
2 1 43210 1  1 2  2 3 18 19 0 42 34 51
3 1 43210 1  1 2  3 4 19 20 0 34 43 52
4 1 43210 1  1 2  4 5 20 21 0 43 35 53
5 1 43210 1  1 2  5 6 21 22 0 35 44 54
6 1 43210 1  1 2  6 7 22 23 0 44 36 55
7 1 43210 1  1 2  7 8 23 24 0 36 45 56
8 1 43210 1  1 2  8 9 24 25 0 45 37 57
9 1 43210 1  1 2  9 10 25 26 0 37 46 58
10 1 43210 1 1 2  10 11 26 27 0 46 38 59
11 1 43210 1 1 2  11 12 27 28 0 38 47 60
12 1 43210 1 1 2  12 13 28 29 0 47 39 61
13 1 43210 1 1 2  13 14 29 30 0 39 48 62
14 1 43210 1 1 2  14 15 30 31 0 48 40 63
15 1 43210 1 1 2  15 16 31 32 0 40 49 64
16 1 43210 1 1 2  16 1 32 17 0 49 33 65
C
17 1 43210 2  3 3  11 7 15 3
C
18 6 30100 14 4 0 1 17 33
19 6 30100 13 4 0 3 19 34
20 6 30100 13 4 0 5 21 35
21 6 30100 13 4 0 7 23 36
22 6 30100 13 4 0 9 25 37
23 6 30100 13 4 0 11 27 38
24 6 30100 13 4 0 13 29 39
25 6 30100 13 4 0 15 31 40
C
26 6 30100 13 4 0 2 18 42
27 6 30100 13 4 0 4 20 43
28 6 30100 13 4 0 6 22 44
29 6 30100 13 4 0 8 24 45
30 6 30100 13 4 0 10 26 46
31 6 30100 13 4 0 12 28 47
32 6 30100 13 4 0 14 30 48
33 6 30100 13 4 0 16 32 49
C
C
BEAMS
SECTION.NUMBER MATERIAL.NUMBER AREA
12 12 0.001
C
C
MESH
REFERENCE SPACING.LIST
1 1
2 6
3 4
4 6
C
C
PLATES.AND.SHELLS
PLATE.OR.SHELL.NUMBER MATERIAL THICKNESS
1 11 150E-06
2 12 750E-06
C
C
SPRINGS
NUMBER.OF.SPRING AXIS.NUMBER KX KY KZ
13 1 500 500 500
14 1 80000 80000 80000
C 14 FOR MANUBRIUM SPRING CONSTANT
C SHOULD BE APPROX. 80000 WITH ONE SPRING
C
MATERIALS
MATERIAL.NUMBER E NU RO MU
C 11 MU 1.5 TO 2.5E-05 DENSITY 1100
11 1.5E06 0.33 1100 0.005
12 1.5E08 0.33 1100 0.005
C
C
```

```
RESTRAINTS
NODE.NUMBER PLANE AXIS.NUMBER DIRECTION
17 1 3 123
C
C
MODES.AND.FREQUENCIES
C AUTOMATIC.MASTERS MODES
C 30 3
MODES
3
C
C
MASTERS
NODE.NUMBER PLANE AXIS.NUMBER DIRECTION
41 0 1 0
C
C
FREQUENCIES.FOR.ANALYSIS
TYPE START FINISH STEP
1 0 1000 0.1
C
C
OUT.DRAW
PLOT.TYPE CASE.NUMBER
C 2 1
1 1
1 2
1 3
C
C
CONTROL
CONTROL.END
C
END.OF.DATA
```

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