Pharmacologic Protection from Noise Induced Hearing Loss: (NIHL): Current Status

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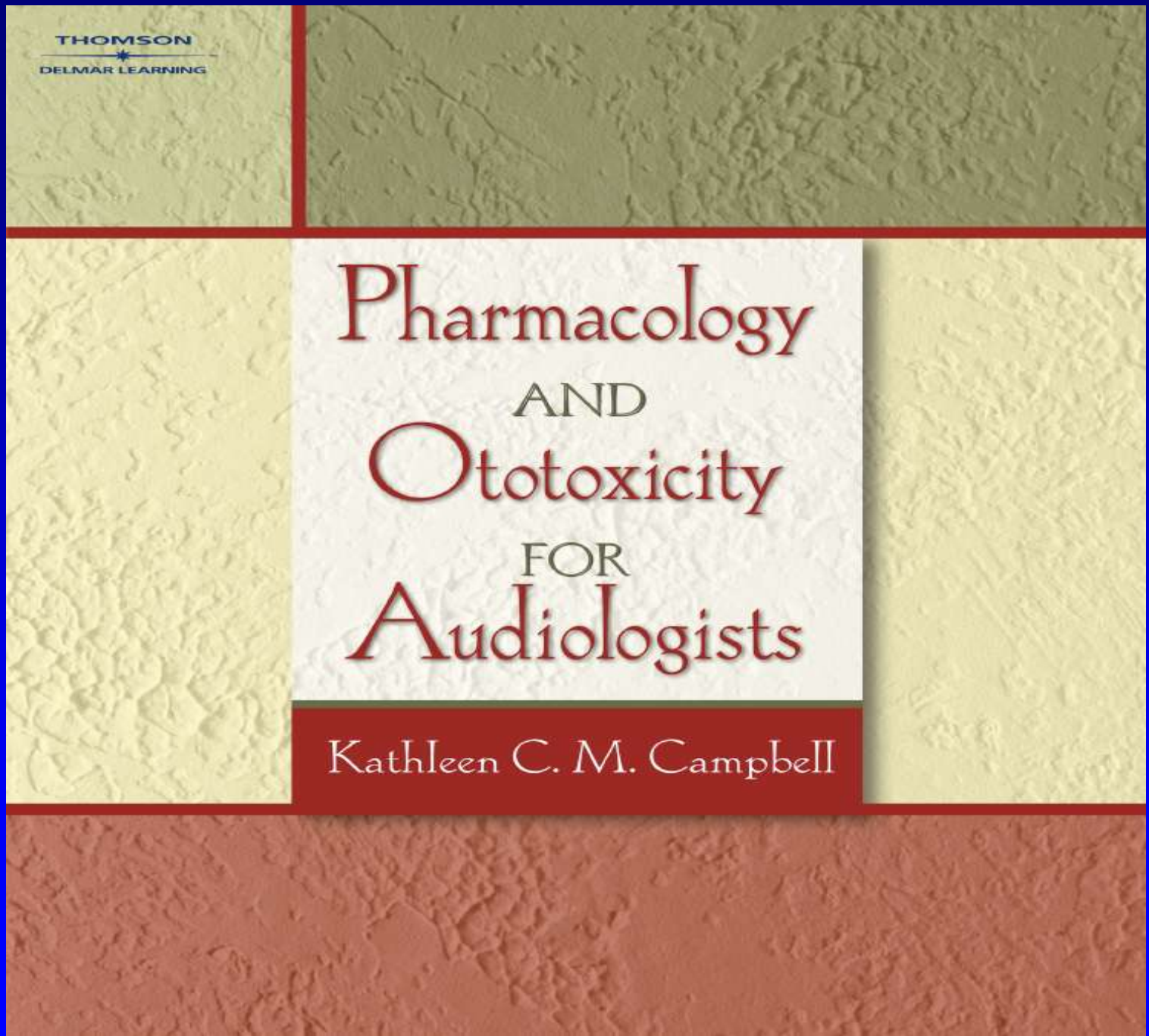

THOMSON
DELMAR LEARNING
Pharmacology
AND
Ototoxicity
FOR
Audiologists
Kathleen C. M. Campbell

Reasons for Developing Pharmacologic Otoprotectants for NIHL

- Even with maximal physical hearing protectors NIHL can occur (bone conduction of high intensity signals)
- Compliance with physical hearing protectors is imperfect
- Noise reduction at the source is not always feasible
- Noise exposure is sometimes unpredictable

Types of Otoprotectants

- Prophylactic Agents: Administered before and usually during noise exposure periods
- Pre-loading has not yet been tested
- Rescue Agents: First administered after noise exposure but before permanent NIHL has occurred
- Regeneration of Hair Cells for permanent NIHL is a different research area

Why Intersubject Variability in NIHL?

- Endogenous Factors:
- Lipid Disorders
- Diabetes
- Reaction to Stress
- Body Fat
- Possible Pigmentation Factors
- Genetic susceptibility and resistance

Why Intersubject Variability for NIHL?

- Exogenous Factors:
- Dietary differences among subjects (eg fat intake, type of fat intake, antioxidants in diet, protein, vitamins, minerals)
- Possible alcohol consumption differences
- Smoking
- Exercise and body fat
- Stress
- Use of Hearing Protectors
- Drugs/medications

Ways to Reduce NIHL?

- Diet (food and alcohol intake, vitamin and mineral intake)
- Exercise and reduction of body fat
- Not smoking
- Stress reduction?
- Hearing protectors
- Diabetes and lipid disorder prevention

When Could a Pharmacologic Agent Prevent/Reduce NIHL?

- When noise exposure exceeds physical hearing protection capability
- When NIHL is secondary to cochlear metabolic damage, not primarily cochlear mechanical damage
- When it can be administered before or within a few days after noise exposure

Reasons Some Agents Are Not Being Developed for the Clinic

- Administration Issues (ie only effective with round window or iv administration)
- Purpose is to elucidate mechanisms of NIHL
- Side Effects
- Not safe in humans (or dosing issues)
- Cost issues
- No patent coverage

Considerations for a Clinically Useful Pharmacologic NIHL Otoprotective Agent

- Oral administration preferable (& palatable)
- Safe (risk/benefit)
- Wide Therapeutic Index
- Minor or no side effects for all subjects
- Minor or no drug interactions
- Easy storage (temperature, volume, preparation, stable over time)
- Preferably Inexpensive

FDA Issues

- Currently no drug is approved by the FDA to treat or prevent noise induced hearing loss (or cisplatin or aminoglycoside induced hearing loss.)
- To obtain FDA approval for a new drug generally takes several years and over 1 billion dollars.
- Therefore patent protection is needed.

Why Focus on Anti-Oxidants for Hearing Protection?

- Many have good safety profiles.
- We know a lot about many of them, sometimes from the nutrition literature.
- Many can be given orally.
- Many of them are not foreign to the human system.
- Side effects are frequently minimal.

How Do Antioxidants Differ From Each Other?

- Mechanisms of action can be different
- They can act on different pathways and/or different cellular areas
- Uptake into tissues and distribution through the body can be different (BLB, BBB)
- Safety profiles can vary (by pt, by drug interactions) Not all work the same on all patients
- Therapeutic indices can vary

What is an Anti-Oxidant?

- A direct anti-oxidant is a compound that can freely donate an electron to stabilize the free radical.
- An indirect anti-oxidant promotes production of endogenous anti-oxidants such as glutathione, or enzymes with anti-oxidant actions (SOD, CAT, GR, GSH-Px)

What is a Free Radical?

- It is an atom, molecule or ion with an unpaired electron on its outer shell. The unpaired electron makes it highly reactive and thus potentially damaging to surrounding molecules.

Glutathione

- A tripeptide consisting of glutamate, cysteine and glycine
- Present in virtually all mammalian tissues and at lower levels in plasma
- Present in reduced (GSH) and oxidized (GSSG or glutathione disulfide) form
- In normal systems 99% of glutathione is in the GSH form

Glutathione

- Altered GSH homeostasis implicated in many disorders (eg Parkinson's)
- Decreased GSH can increase therapeutic efficacy of some drugs and radiation but can increase side effects (ie can increase toxicity for all cells)
- Helps eliminate xenobiotics
- Important antioxidant pathway (reductant)

GSH Antioxidant Role

- Essential component of antioxidant defenses
- Protects cells from oxidative damage by donating a hydrogen atom from the thiol group of the cysteine residue
- Hydrogen atom can be donated to most carbon, nitrogen, or oxygen centered radicals

Cellular GSH

- Most cellular GSH is in the cytosol
- Only 10-20% of GSH is in the mitochondia

Enzyme

- Complex protein substance produced in living cells
- Causes or accelerates other chemical reactions in an organism
- Is not altered itself
- Enzymes are organic catalysts

Two enzymes you should know

- Superoxide Dismutase: Converts $O_2^{\bullet}$, the superoxide radical anion, into oxygen (O_2) and hydrogen peroxide (H_2O_2) .
- Catalase: Converts hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2).

Two More Enzymes You should Know

- Glutathione Peroxidase oxidizes GSH to GSSG
- Glutathione Reductase reduces GSSG to GSH

Natural Otoprotective Agent

- Axelsson 1975: Pop/rock musicians had lower than predicted levels of hearing loss
- Liking the music?
- Continuous contraction of stapedius muscle/
- Efferent system does not have a major protective effect (Liberman and Gao 1995)

Moderate alcohol consumption

- Moderate alcohol consumption (less than 4 drinks per day) inversely correlates with the odds of having LF or HF NIHL (Popelka et al 2000)
- Heavy alcohol consumption increased the odds of HFHL

Putative Protective/Recue Agents for NIHL

- GSH Prodrugs
- Magnesium
- NOS Inhibitors
- Cell Death Inhibitors
- Free Radical Scavengers/Antioxidants
- Antioxidant enzyme protectants/upregulators
- Combinations?

Why Not Just Administer Glutathione Directly?

- Intracellular GSH is probably a major factor in cochlear protection
- However the liver metabolizes GSH and it is not readily taken up into cells (Meister 1991)
- GSH esters may produce toxicities (Levy et al 1993)
- RW application effective but not practical (Hight et al 2000)

Neurotrophic Factors

- Some neurotrophic factors show good NIHL protection (eg glial cell line-derived neurotrophic factor Ylikoski et al 1998, Shoji et al 2000) but may be secondary to anti-oxidant properties
- Neurotrophic factors without anti-oxidant properties (brain derived neurotrophic factor, fibroblast growth factor) may not protect (Miller and Altschuler 2000, Shoji et al 200)
- Oral availability and safety may also be issues

Dietary Supplements

- Several NIHL otoprotective agents are also micronutrients:
- Mg: fish, almonds, spinach, shrimp, bran
- D-met: cheese, yogurt
- NAC: brussel sprouts
- Resveratrol: red wine
- Selenium: Brazil nuts, N. Dak and S.Dak grown foods, prime component of ebselen
- Alcohol: 2-4 drinks per day

Antioxidant therapies Approaching Clinical Trials

- Ebselen
- N-Acetylcysteine (NAC)
- D-Methionine (D-MET)
- ACE Mg
- Salicylate (as concomitant agent)

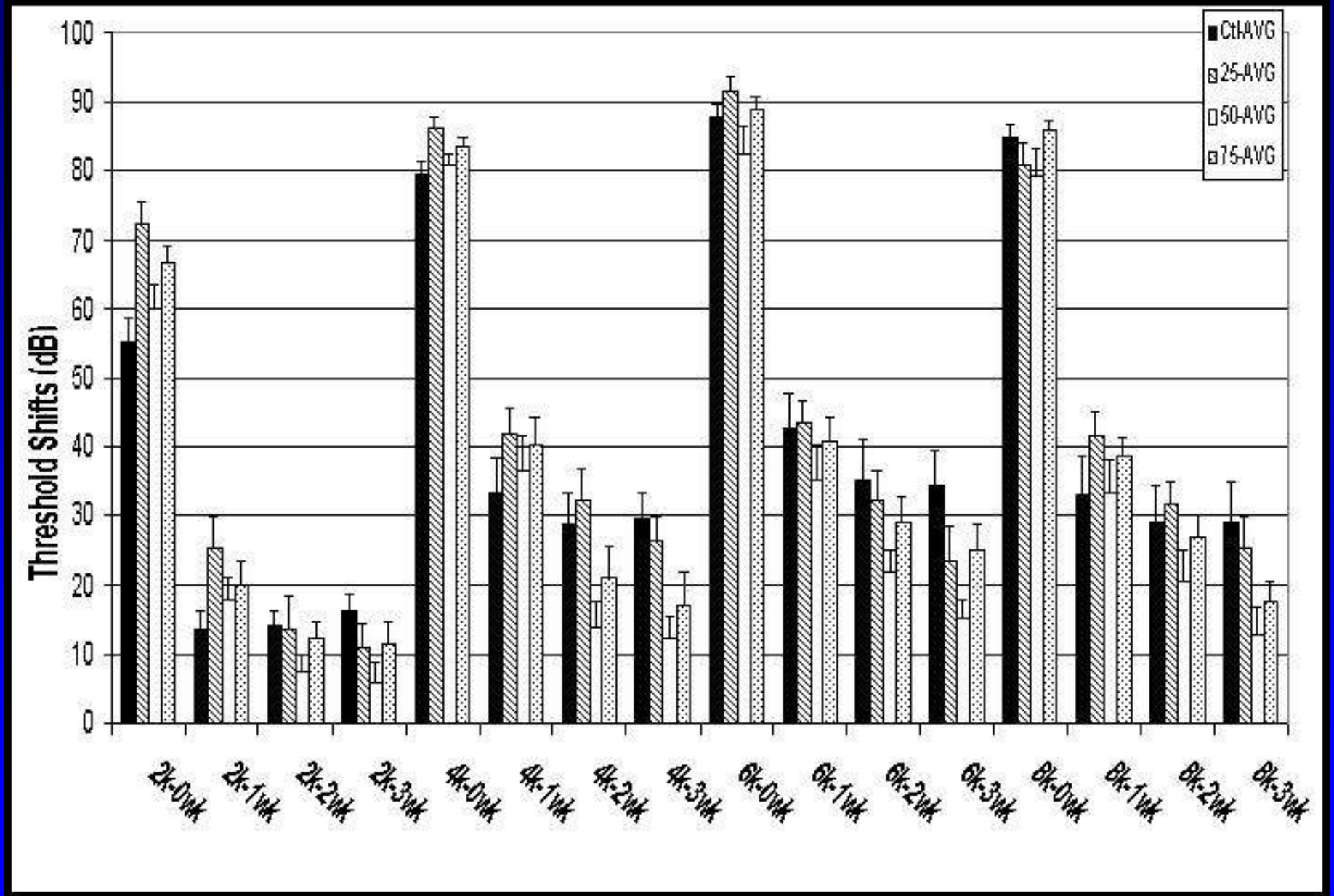
Agents have good safety profile and oral bioavailability

Kopke et al 2000, 2002

- Chinchilla model
- 105 dB SPL noise band centered at 4kHz
- D-met or ALCAR administered at 200mg/kg ip, NAC at 325mg/kg plus salicylate
- Administered every12 hours starting 48 hours prior to noise and 1 hour prior to the noise and then twice per day for 2 days following noise exposure

N-acetylcysteine (NAC): putative mechanisms

- L-NAC is a free radical scavenger
- Neuroprotectant
- GSH precursor: provides cystolic but not mitochondrial GSH


Threshold Shifts (dB)
100
90
80
70
60
50
40
30
20
10
0
CtlAVG
25-AVG
50-AVG
75-AVG
2k-0wk
2k-1wk
2k-2wk
2k-3wk
4k-0wk
4k-1wk
4k-2wk
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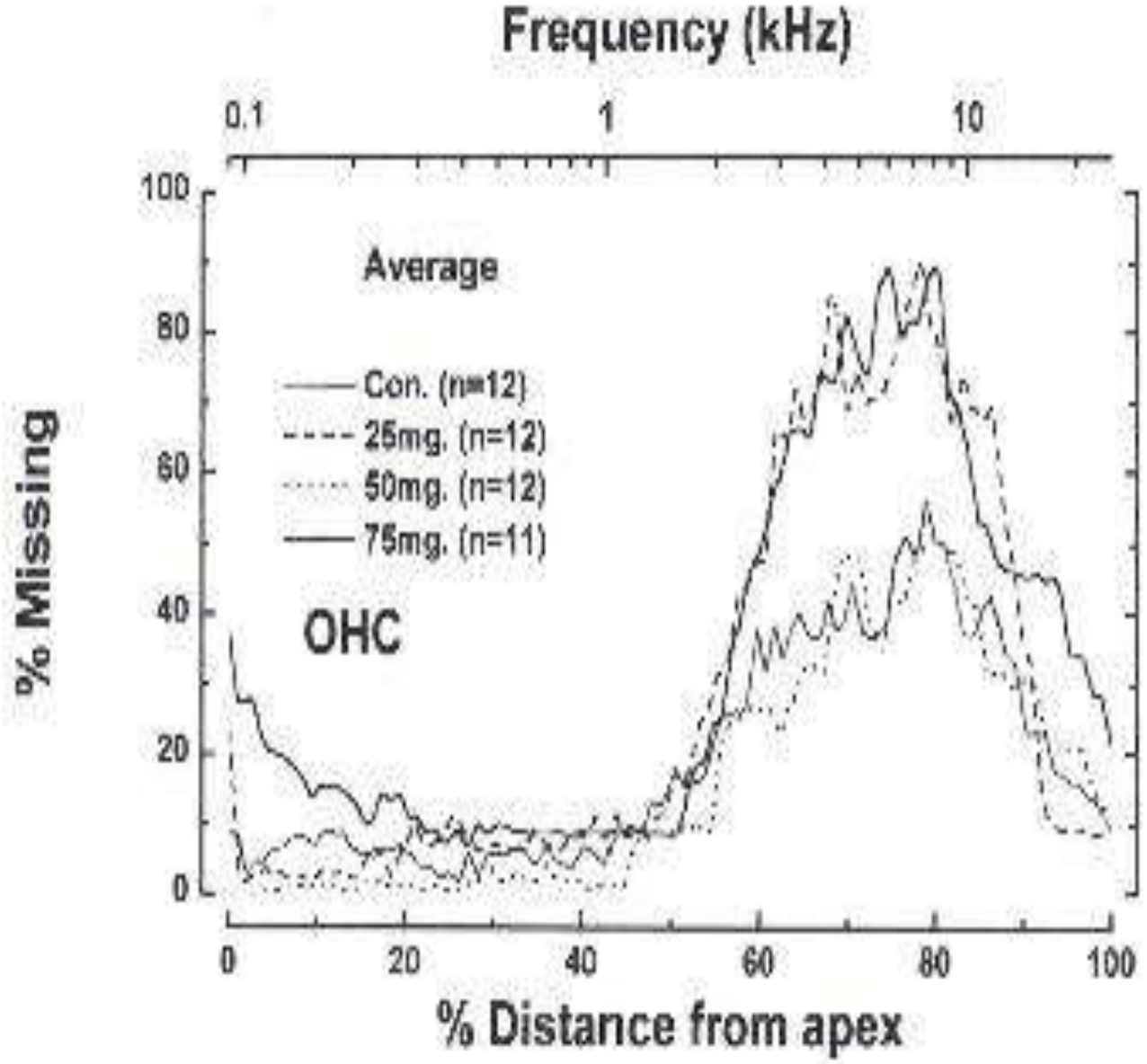
8k-0wk
8k-1wk
8k-2wk
8k-3wk


Frequency (kHz)
0.1
1
10
Average
Con. (n=12)
25mg. (n=12)
50mg. (n=12)
75mg. (n=11)
OHC
% Missing
100
80
60
40
20
0
0
20
40
60
80
100
% Distance from apex

Ebselen

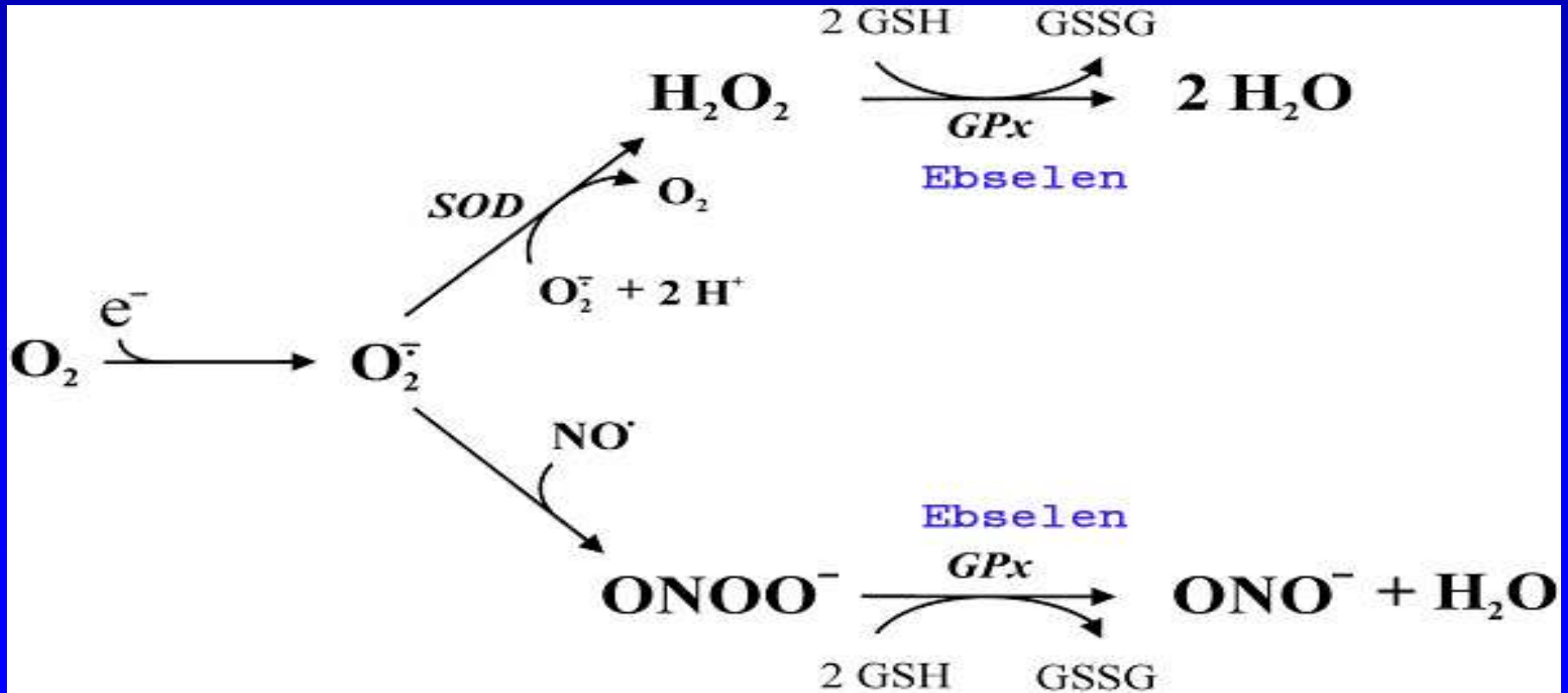
A Catalyst for Hearing Loss Treatment

Eric D. Lynch, PhD

4010 Stone Way N Suite 120
Seattle, WA

Ebselen (SPI-1005)—How does it work?

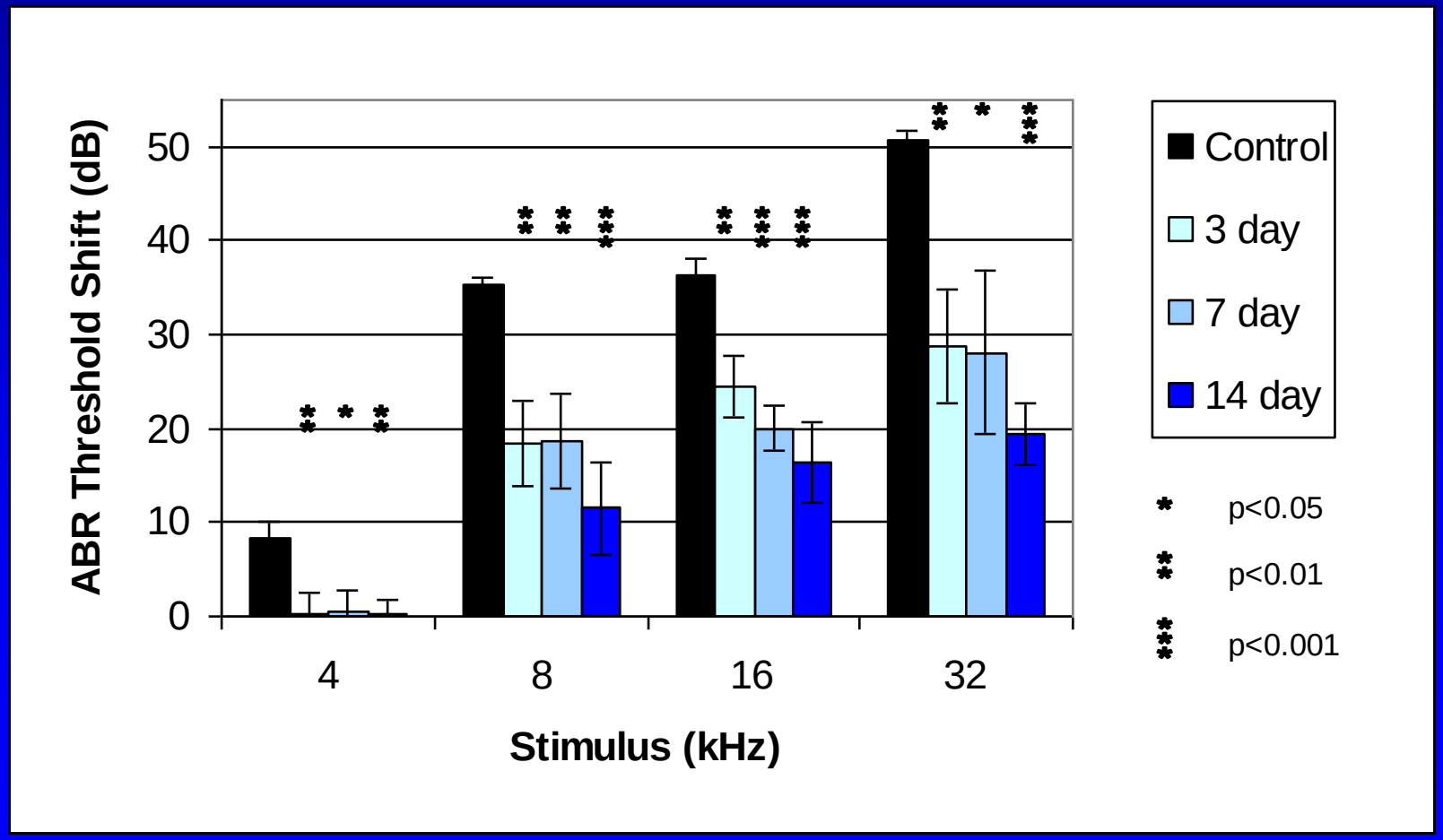
- Small molecule mimic of glutathione peroxidase (GPx)
  - Glutathione Pathway—ROS/RNS neutralization
  - GPx catalytic activity



Otoprotection across frequencies

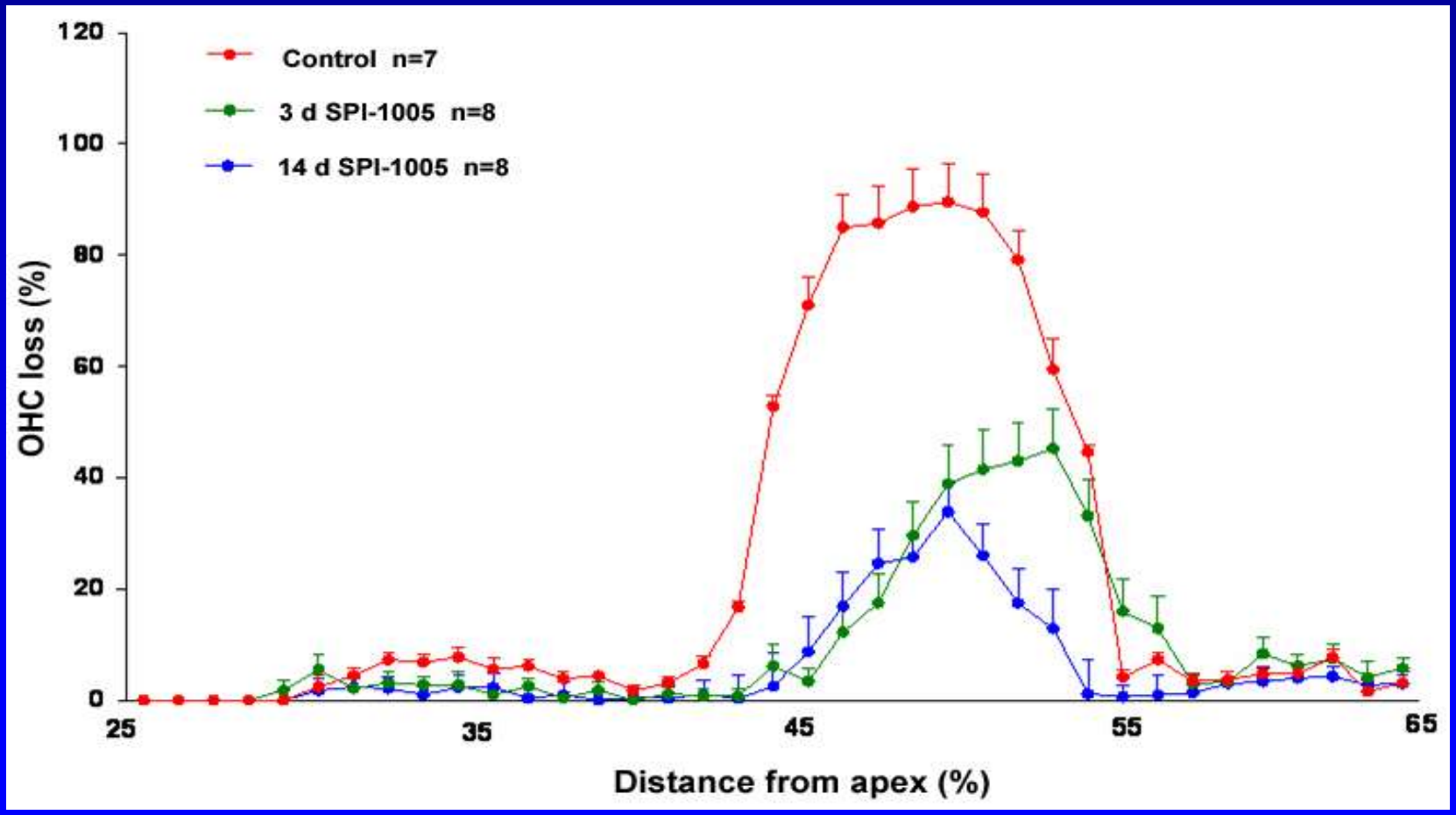
Continuous 4 hr noise exposure
4-16 kHz noise at 113 dBSPL

4 mg/kg SPI-1005, ABR at 9 wks post noise, n=8 (3 & 14d), n=6 (7d), SEM shown



Cytocochleogram analysis

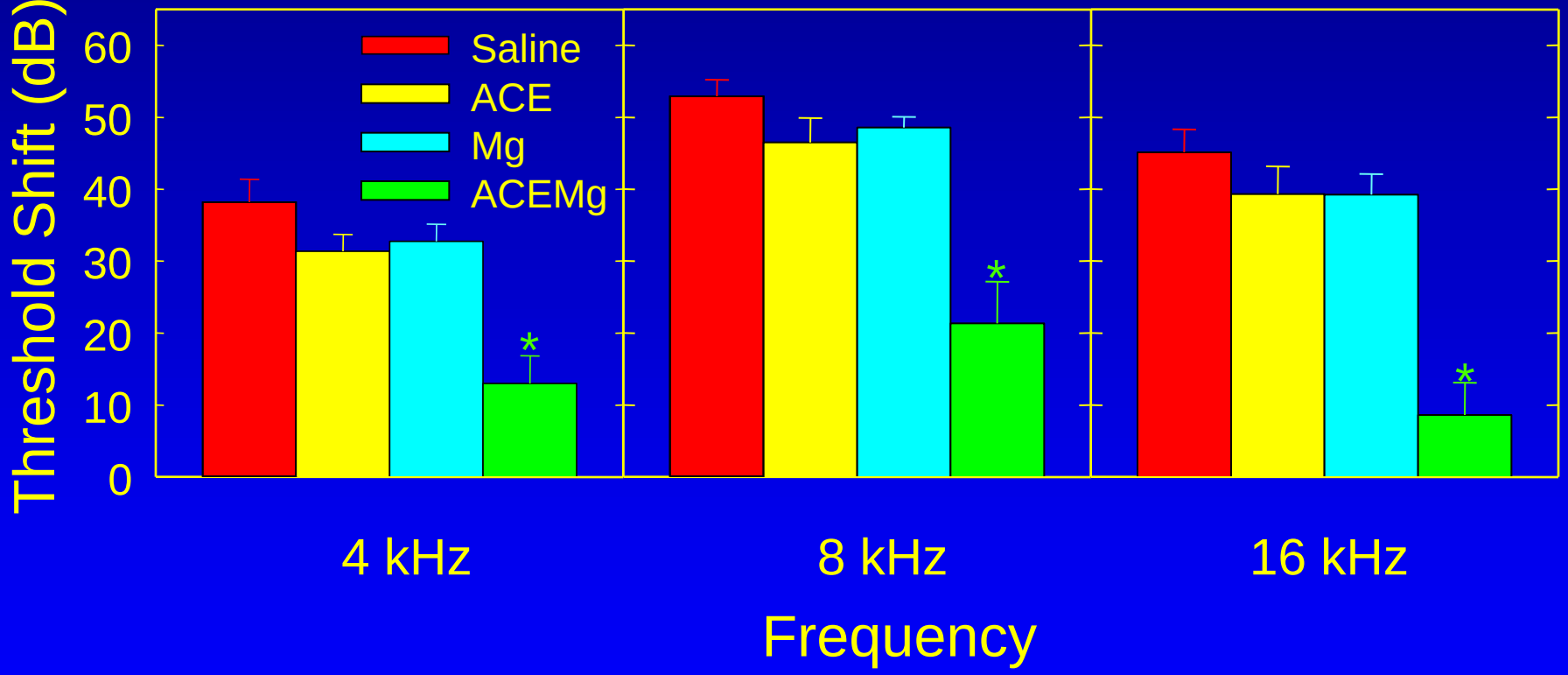
3 wks post noise



Dietary Micronutrients

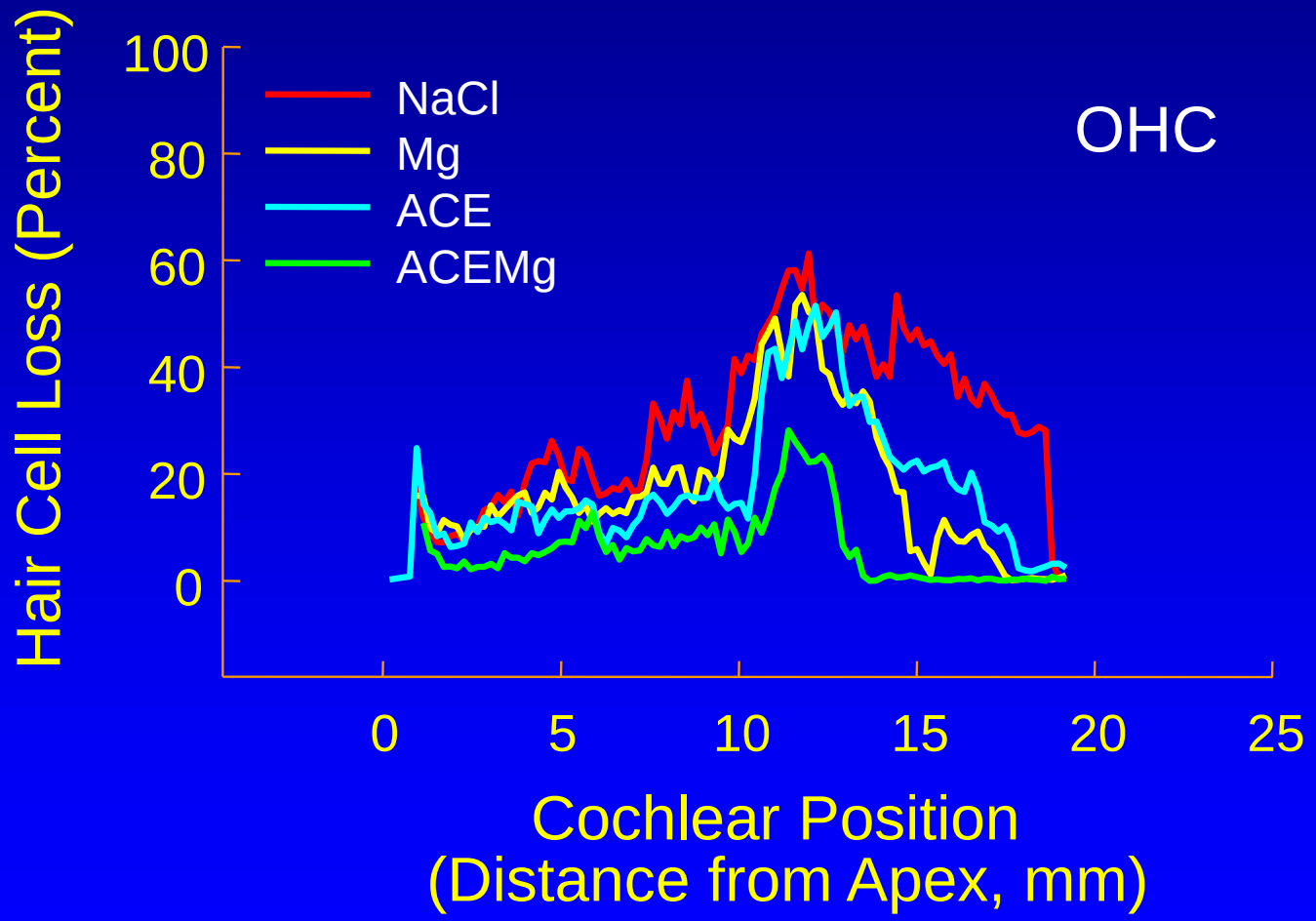
- Beta-carotene, Vitamins C and E, Magnesium
  - Beta-carotene: scavenges singlet oxygen, prevents lipid peroxidation
  - Vitamin E: reduces peroxyl radicals in lipid layer
  - Vitamin C: scavenges free radicals in aqueous phase
  - Magnesium: reduces noise-induced vasoconstriction, blocks NMDA receptors, prevents calcium influx and neural excitotoxicity
- Patent pending, University of Michigan
  - Inventors: Josef Miller, Colleen Le Prell, Jochen Schacht, Diane Prieskorn
- Option to license by OtoMedicine, Inc.
- Human trials beginning in 2008

Antioxidants plus magnesium reduce noise-induced hearing loss: additive effects



2.1 mg/kg beta-carotene, p.o., 71.4 mg/kg L-threoascorbic acid, s.c., 26 mg/kg trolox, s.c.); magnesium sulfate, 2.85 mmol/kg, equivalent to 343 mg/kg, s.c.; 1 hour pre and 5 days post Mean ± S. E., Le Prell et al., *Free Rad. Med. Biol.*, 42,1454-1463.

Protection is greatest in the base of the cochlea



Human Clinical Trials: 2008-2013

- Temporary Threshold Shift Model
  - Swedish soldiers exposed to automatic weapons fire
  - US students listening to music with insert earphones
- Permanent Threshold Shift Model
  - NATO soldiers at Spanish airbase
  - Employees at Spanish stamping factory

Human Trials

- **Safe dosing limits well-characterized**

	US RDA	Upper Limit	Percent of UL
B-Carotene	1.5 mg/5000 IU (18 mg[1])	3 mg/10,000 IU (36 mg[1])	50% (18 mg) 90% of EU UL
Vitamin C (Ascorbic Acid)	60 mg	2000 mg	25% (500 mg)
Vitamin E (α-tocopherol)	15mg	1000 mg	27% (270 mg)
Magnesium	300-400 mg	350 mg	90% (315 mg)

[1] Based on retinol activity equivalents
EU Upper limit=20 mg

Procedures to be used

- Pure-tone Audiometry
  - Conventional and Extended High Frequencies
- Distortion Product Otoacoustic Emissions
  - Input-Output Functions
- Tinnitus Surveys

Key Steps To Date: Funding

- Funding has been obtained from the NIH
  - Submitted Translational Research Application, Reviewed by Neurology Institute Clinical Trials section, with ad hoc ENT participation
- Parent grant awarded to UM (PI: Josef Miller); Subcontracts to each trial site and other partner institutions

Key Steps To Date: MOP, DSMB, TSB

- Submitted 200+ page "Manual of Procedures (MOP)" for NIH Review, Nov. 2007
- Data Safety Monitoring Board (DSMB) assembled by NIH after review of MOP
- Submitted revised MOP for review by DSMB, Dec. 2007
- Submitted 700+ page "Trial Site Binder (TSB)" for NIH Review, Jan. 2008
- Meeting of study team members with NIH and DSMB took place in Jan. 2007

Key Steps To Date: Additional Administrative Steps

- IRB applications at UM and at each trial site must match, and both must be approved
  - Swedish military trial approved both at UM and by Swedish regulatory committee
- Subcontracts with trial sites under negotiation
- Initial site visits scheduled to occur prior to trial onset

Conclusions

- Antioxidants attenuate NIHL
  - Across species
  - Across agents
  - Across dose schedules
  - Across noise insults
- We can effectively reduce NIHL even with treatments that *follow* the noise exposure
- It is indeed time to evaluate antioxidant prevention of NIHL in human trials

Contraindications for ACE Mg

- Vitamin A and its precursors (Beta Carotene) should not be administered to smokers
- Magnesium has laxative properties and may not be appropriate for subjects with gastrointestinal disorders.

Current Status of D-met Research

- FDA previously approved our Investigational New Drug Application for D-met protection from radiation induced oral mucositis.
- Phase I Clinical Trial Data has been submitted for publication.
- Phase II clinical trials (India) results for protection from cisplatin induced ototoxicity and radiation induced oral mucositis are being prepared for publication.
- In discussions with military for NIHL protection.
- More bench work also needed.

Patents Issued

- Southern Illinois University School of Medicine
- Kathleen C.M. Campbell, PhD Inventor
- 5 US patents and 34 foreign patents issued
- Others in prosecution

Other findings

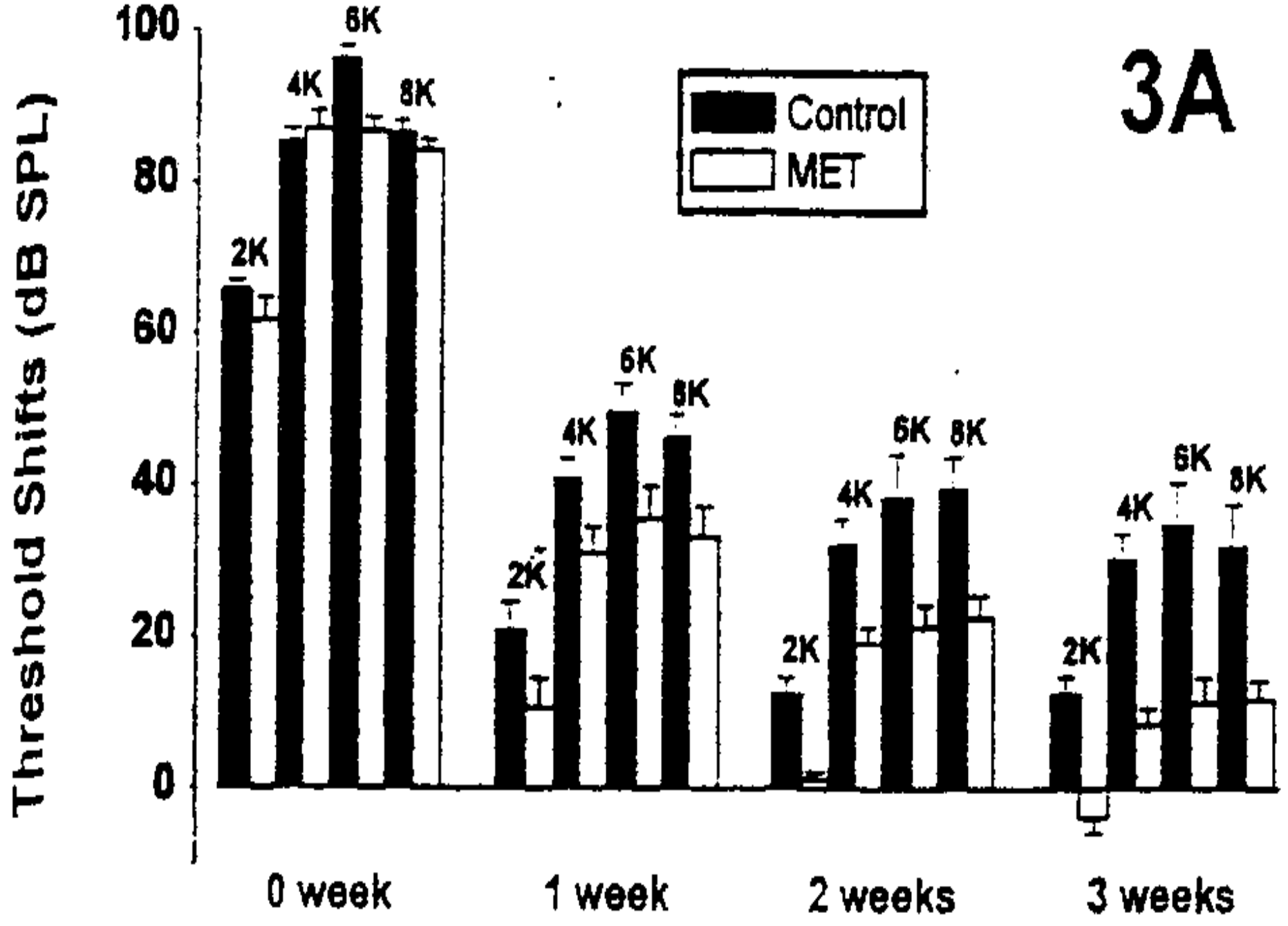
- D-met can be administered directly to the round window and still protect against systemic or topical cisplatin-induced ototoxicity
- D-met protects against carboplatin-induced ototoxicity
- D-met protects against aminoglycoside-induced ototoxicity
- Some patients receive all 3 drugs
- D-met also can protect against NIHL

D-methionine: putative mechanisms

- Unlike most amino acids, methionine is reversibly oxidized (Vogt 1995) and thus may serve as a free radical scavenger
- Methionine can provide cysteine, a precursor for glutathione (GSH) synthesis
- Can increase mitochondrial GSH levels and can prevent the efflux of GSH from injured cell
- May protect antioxidant enzyme levels
- D-met well tolerated even at high dosages

D-methionine: putative mechanisms (continued)

- At least for noise improves/protects GSH/GSSG ratio
- Preferable pharmacokinetics to L-met for protection
- Has been previously used in humans and animals
- Part of normal nutrition (particularly present in fermented proteins)
- Methionine part of protein and needed for protein formation


3A
Threshold Shifts (dB SPL)
100
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Control
MET
2K
4K
6K
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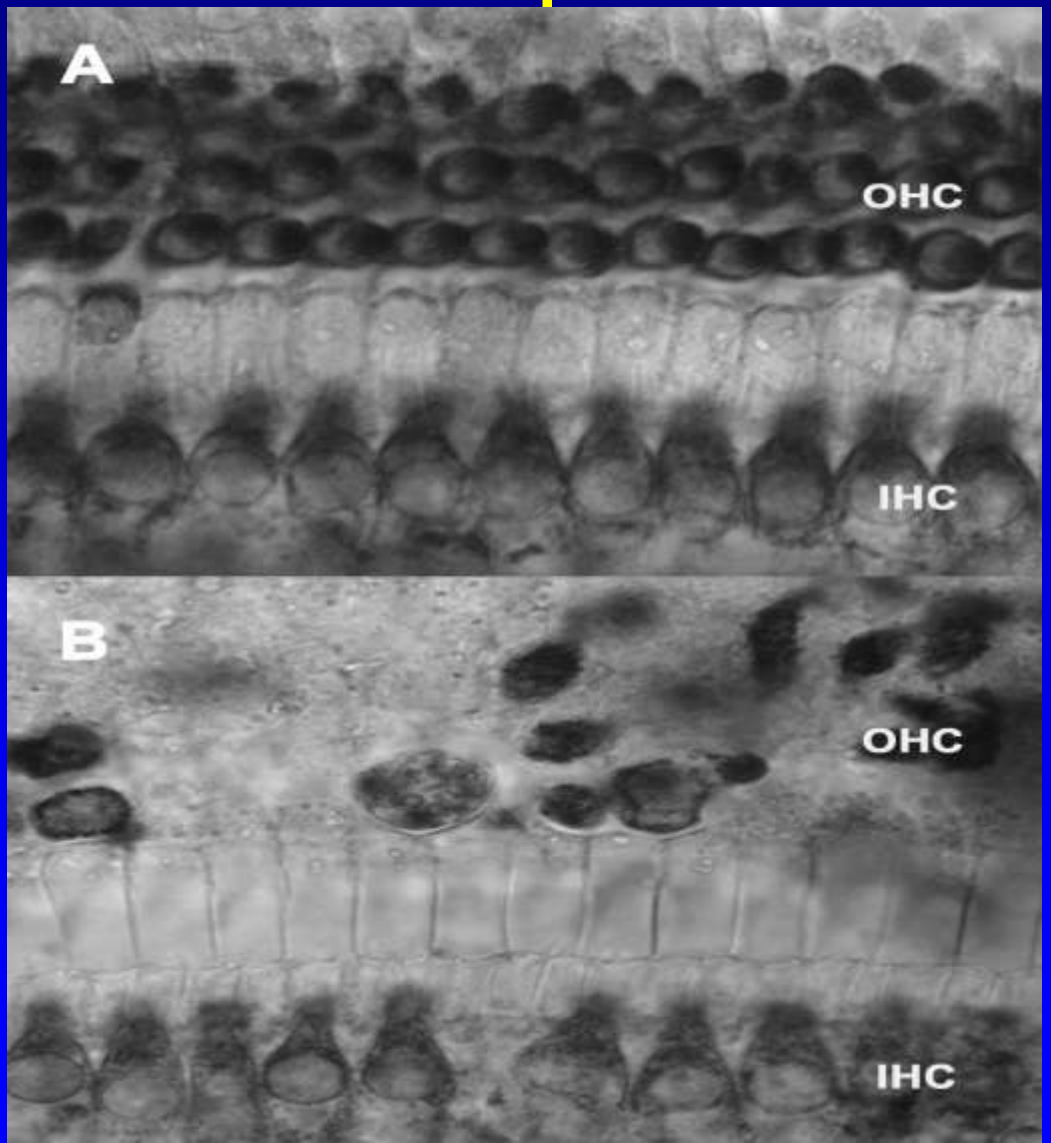
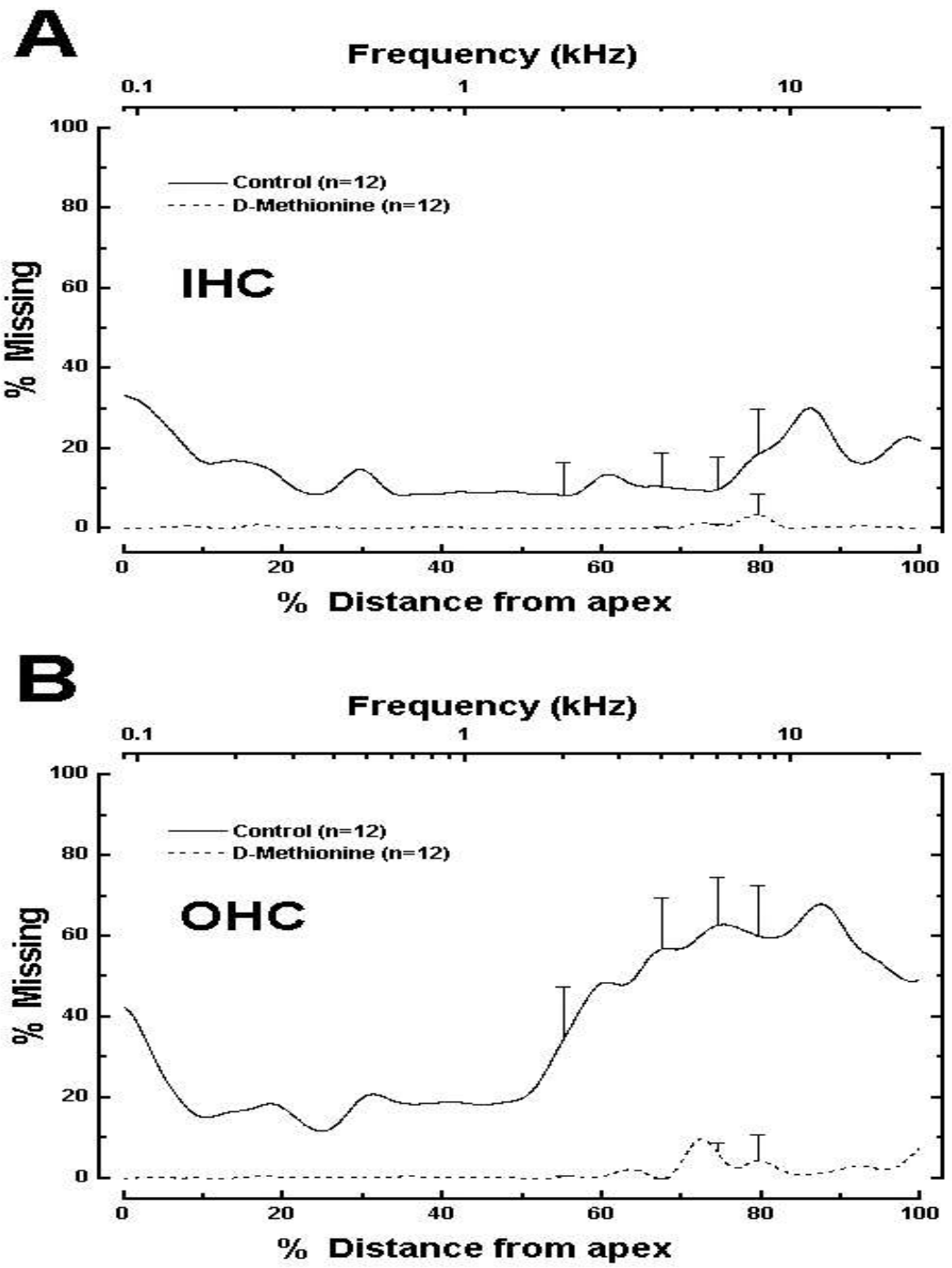
0 week
1 week
2 weeks
3 weeks

Met HC protection




A
Frequency (kHz)
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Control (n=12)
D-Methionine (n=12)
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D-Methionine (n=12)
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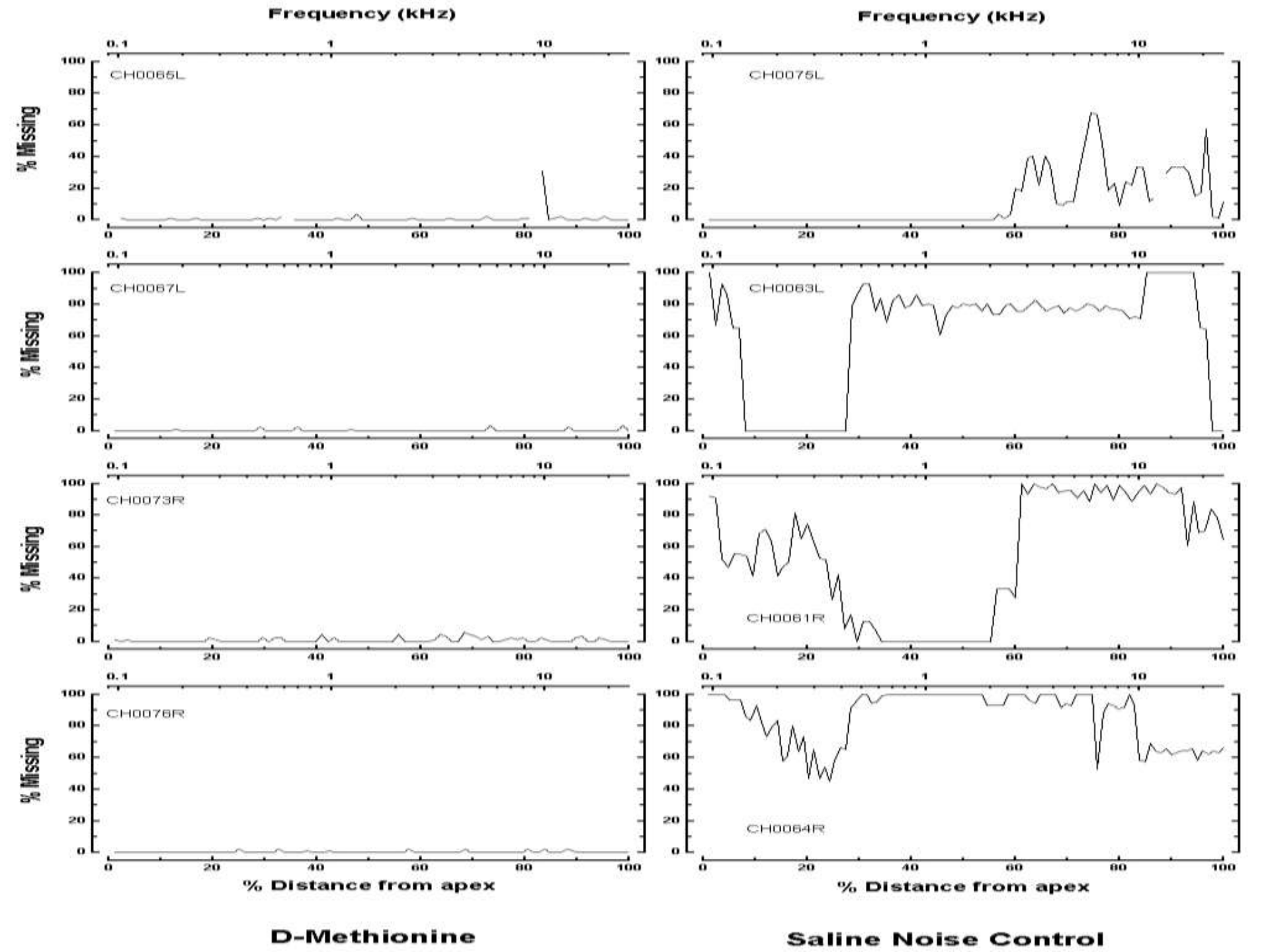
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Frequency (kHz)
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CH0063L
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CH0061R
CH0076R
CH0064R
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20
40
60
80
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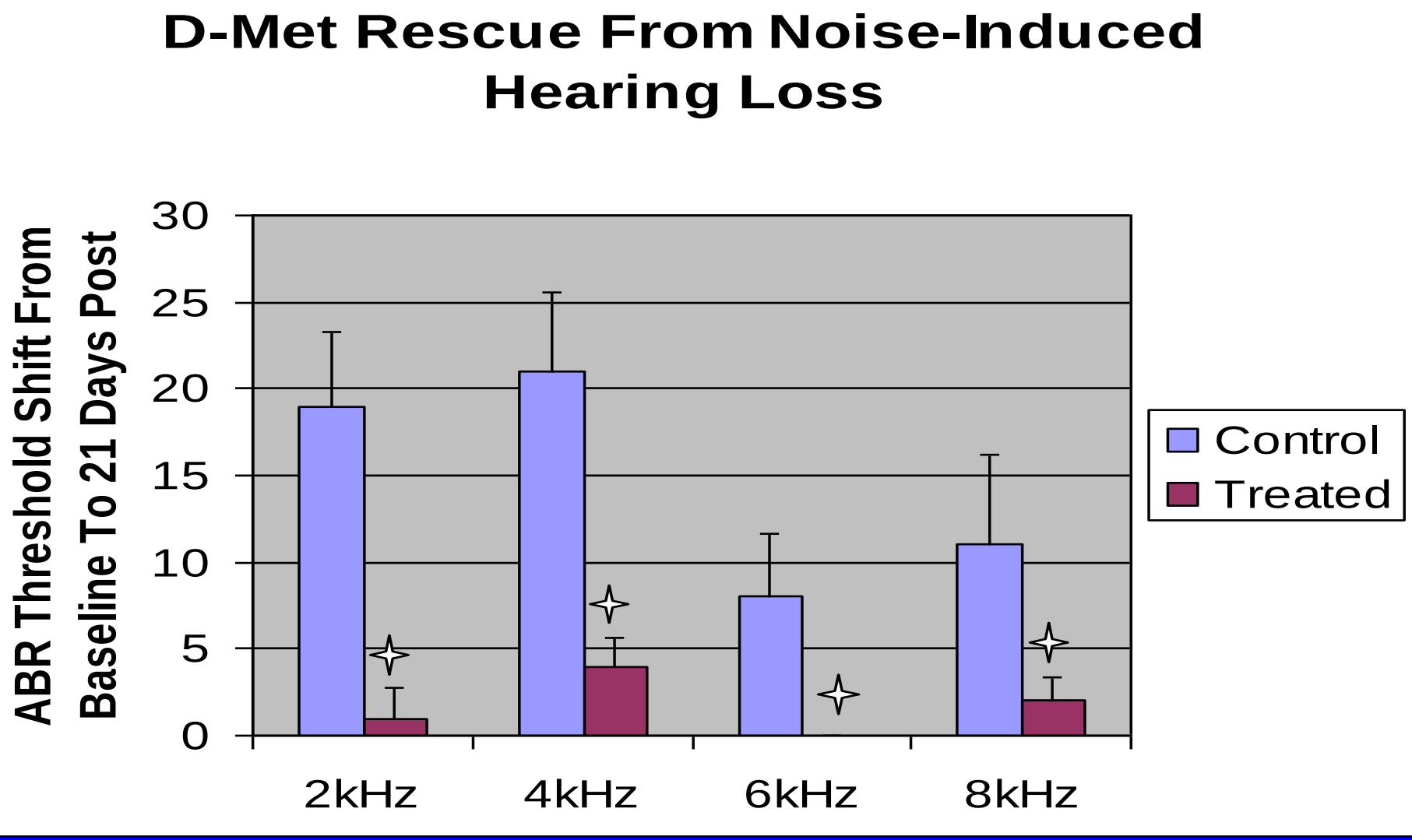
% Distance from apex
% Distance from apex
D-Methionine
Saline Noise Control

D-met Post-Noise Rescue Mitchell, Meech, Campbell

- D-met can be administered 1 hour after noise exposure and provide protection from permanent NIHL.
- Does not provide significant against TTS but only PTS.
- Methods: 6 hour: 105dB SPL 4kHz octave band noise, 200 mg/kg D-met 1 hour after exposure and 2 days BID.
- With 10 animals per group, significant protection at 2, 4, 6 & 8 kHz

D-met rescue from NIHL



D-met Rescue from Noise-Induced Hearing Loss: Post Administration Intervals

Kathleen Campbell, PhD, Robert Meech, MA, Deb Larsen, MA, Diana Mitchell, MD, Larry Hughes, PhD

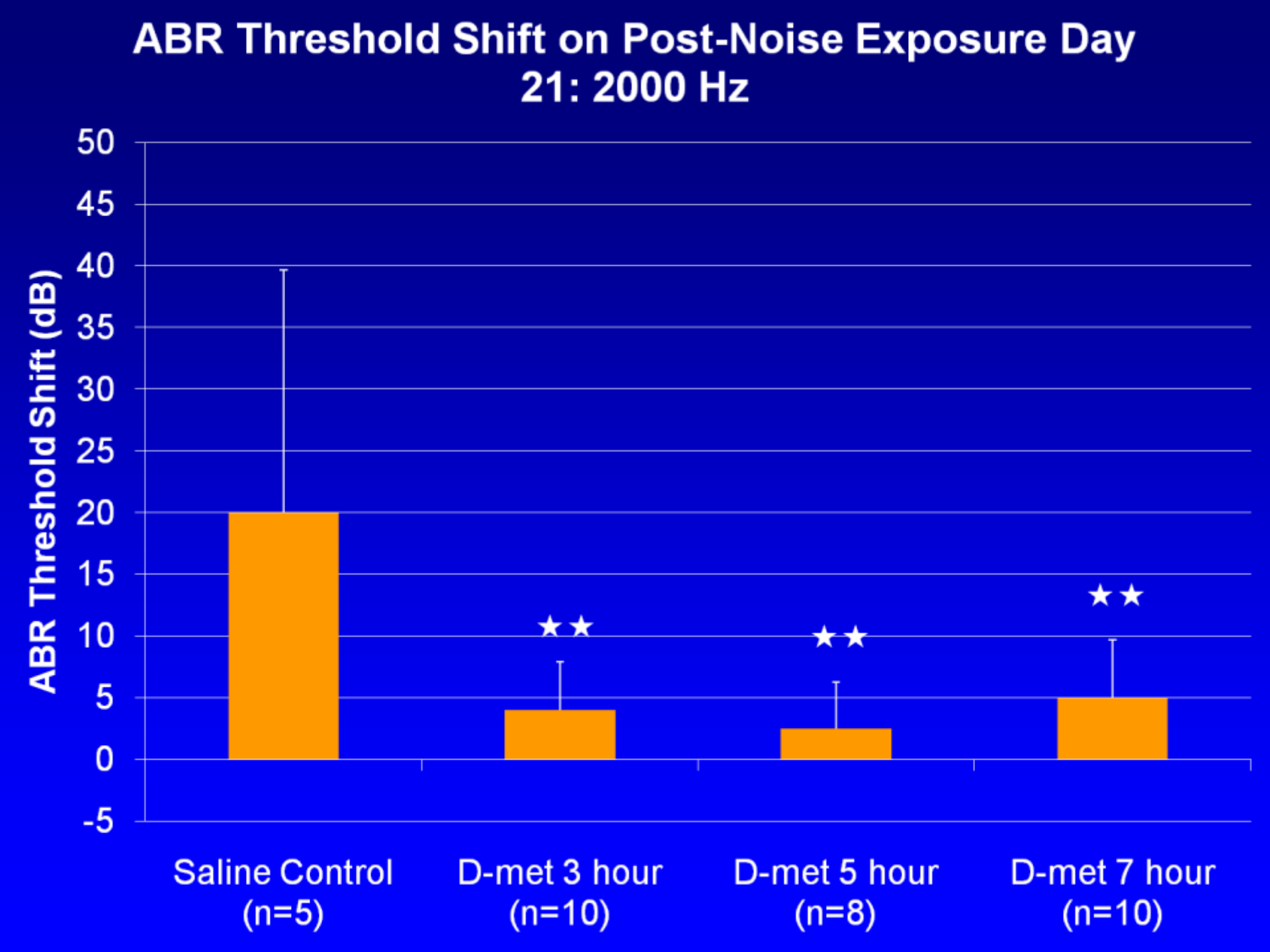
Clinical Question: How long can D-met be delayed and still prevent permanent NIHL?

- Unexpected high level noise exposure can occur in:
- Certain professions such as emergency workers, miners, military personnel
- Recreational exposure such as concerts, power equipment
- Air bag deployment, alarm systems

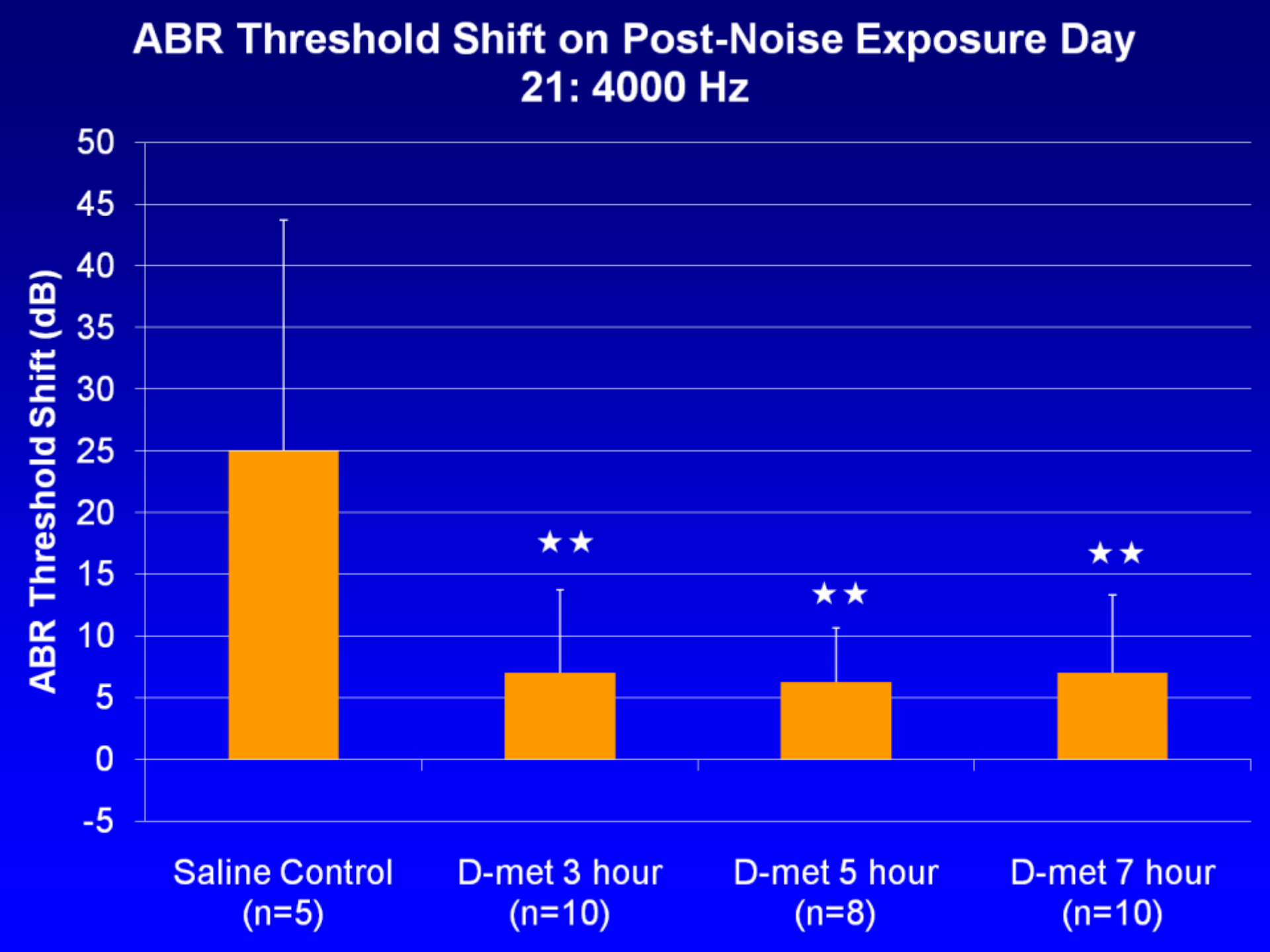
Methods:

- Study in progress
- 105 dB SPL 4 kHz NB for 6 hours
- Chinchillas Laniger (male 3 year old)
- 200 mg/kg D-met initially started at either 1, 3, 5 or 7 hours after noise cessation with 4 additional BID doses 12 hours apart
- 5 animals per group for 1, 3, 5 hour groups and saline control, 3 per group at 7 hours
- ABR thresholds and outer hair cell counts at 21 days

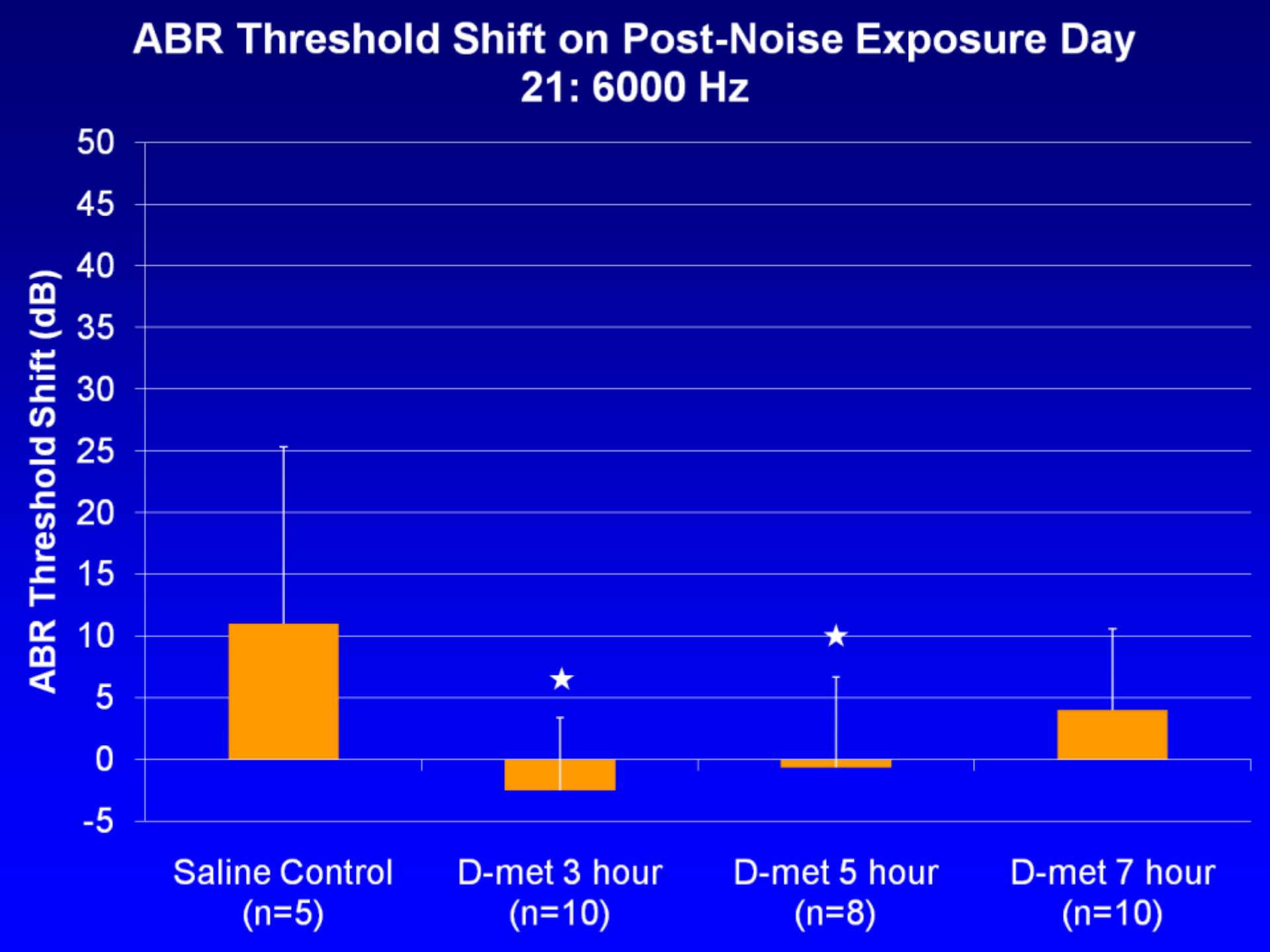
ABR Threshold Shift on Post-Noise Exposure Day 21: 2000 Hz



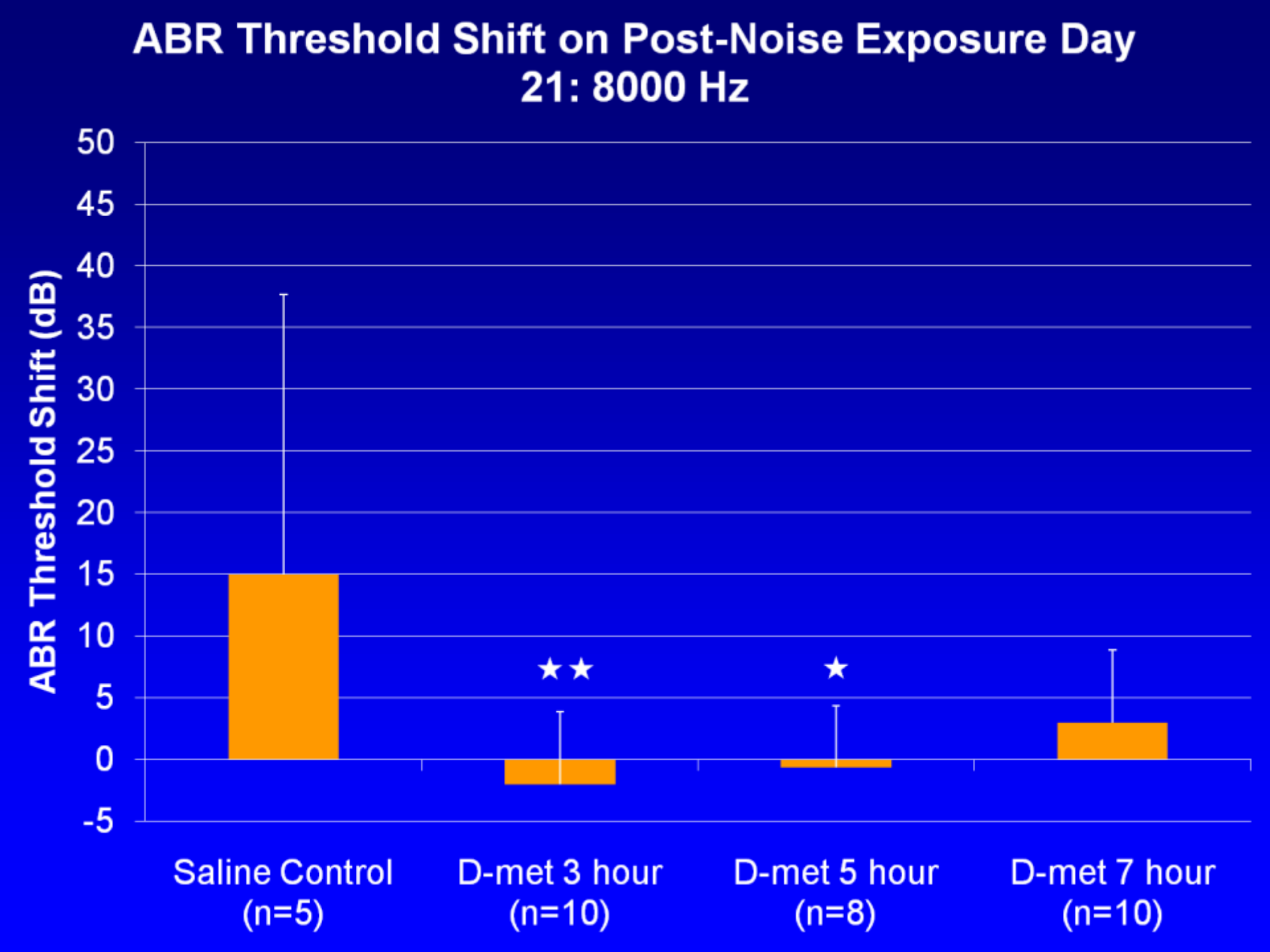
ABR Threshold Shift on Post-Noise Exposure Day 21: 4000 Hz



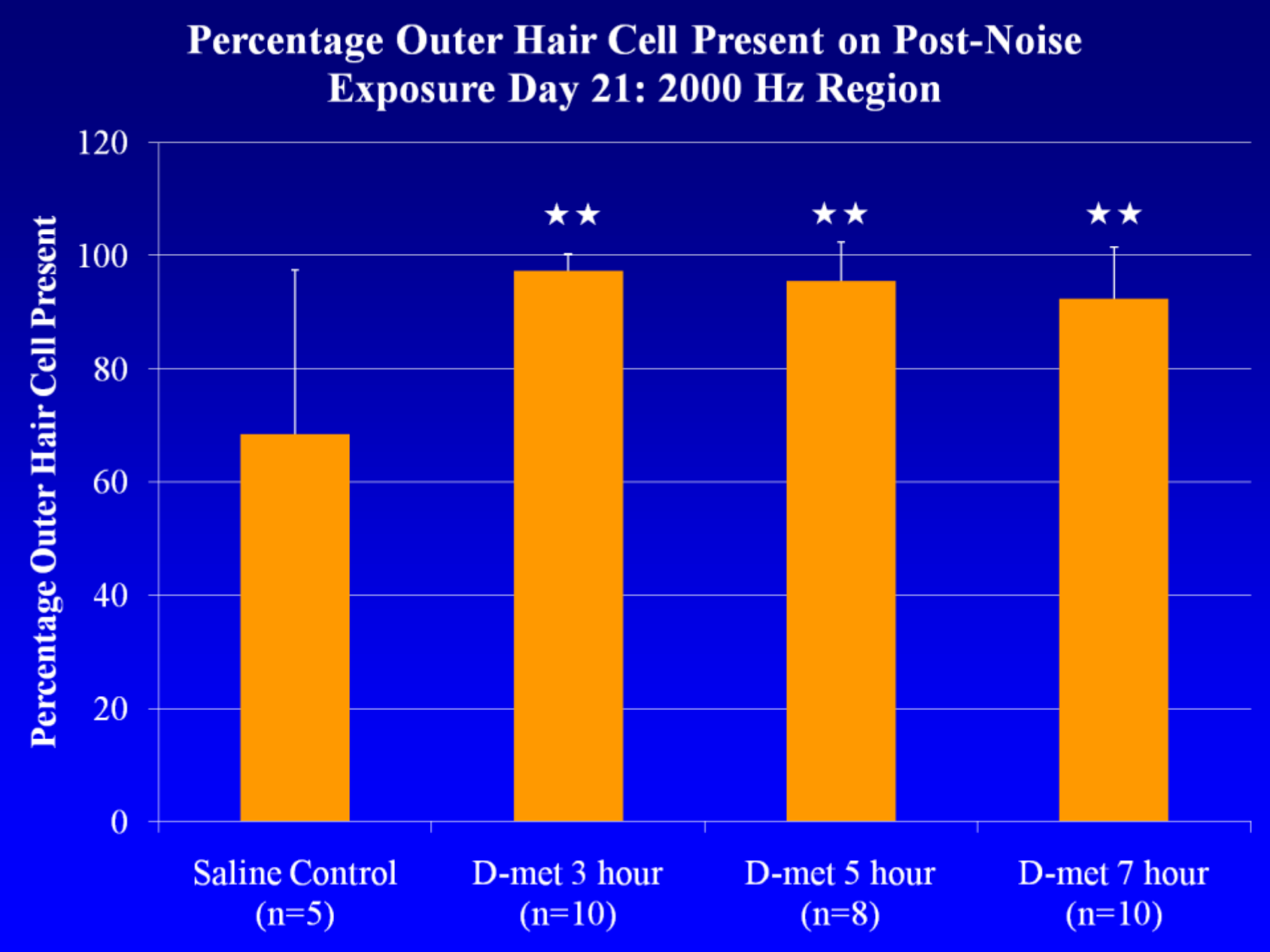
ABR Threshold Shift on Post-Noise Exposure Day 21: 6000 Hz



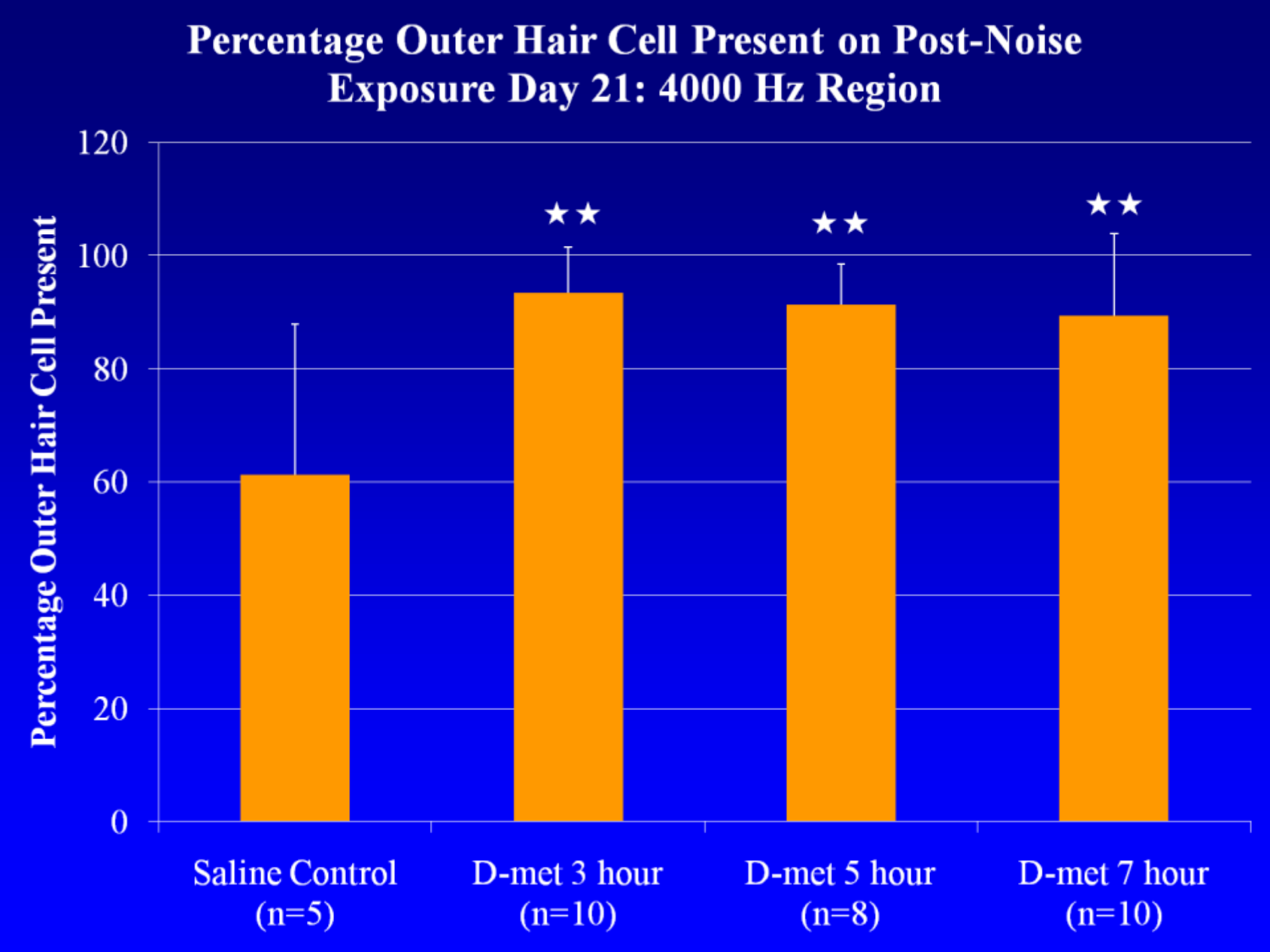
ABR Threshold Shift on Post-Noise Exposure Day 21: 8000 Hz



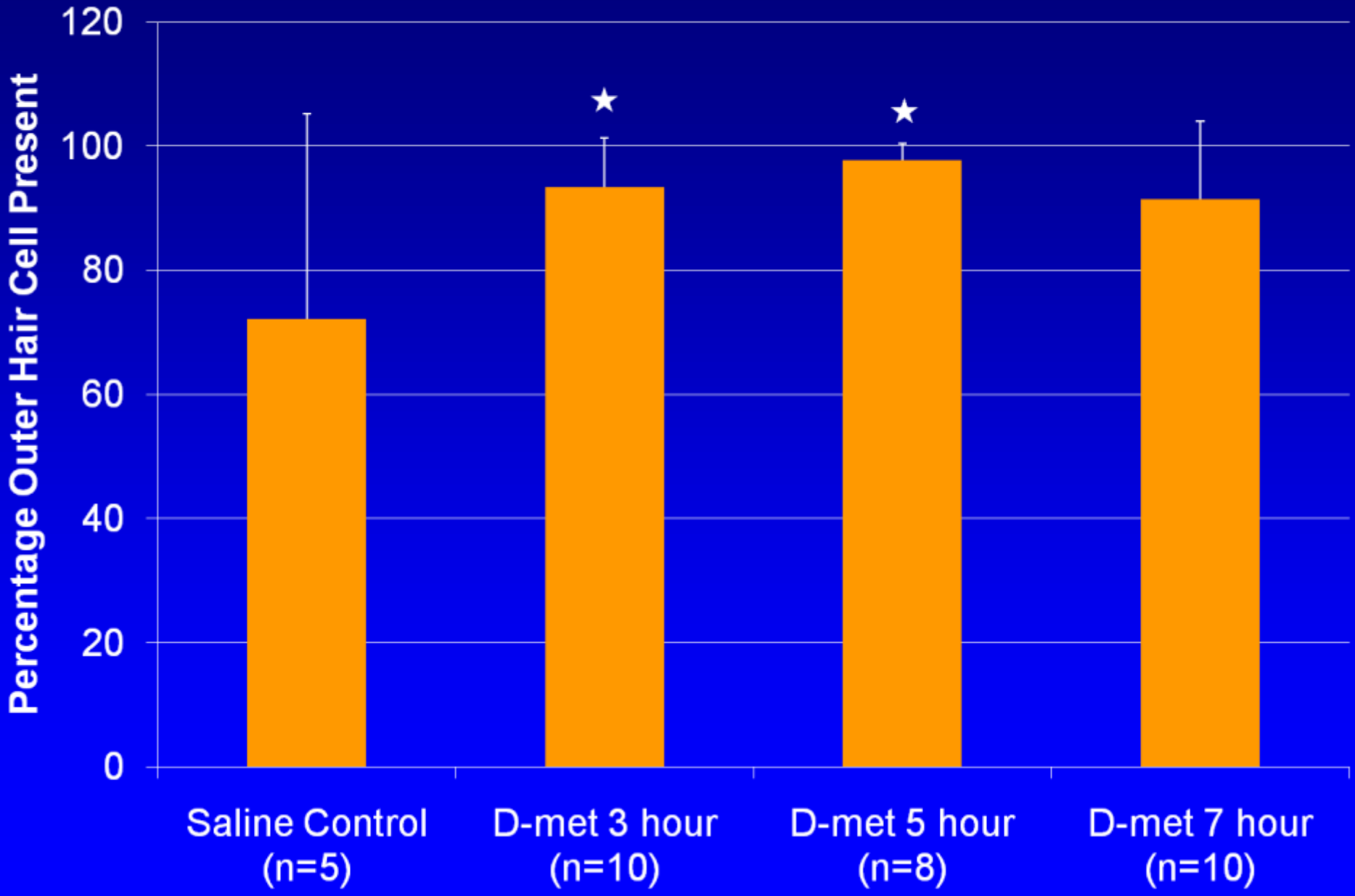
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 2000 Hz Region



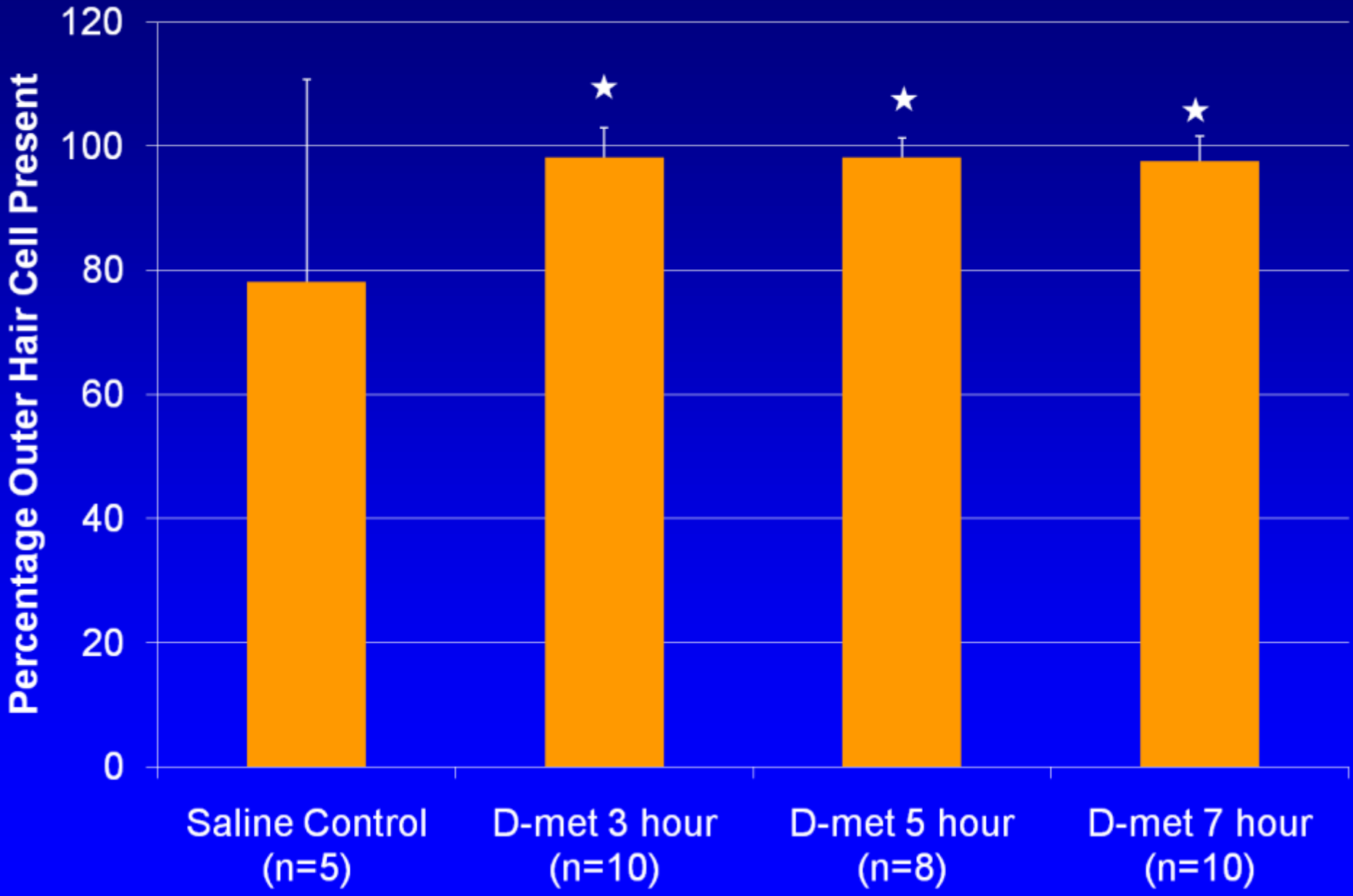
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 4000 Hz Region



Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 6000 Hz Region



Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 8000 Hz Region



Conclusions:

- Post-noise D-met administration, at least within 5 hours following the noise, may protect against permanent ABR threshold shift and OHC loss.
- Further research needed to explore maximum allowable time delay, various D-met dosing strategies, and efficacy for different noise exposures.
- Further research needed on D-met mechanisms of protection for not only NIHL, but aminoglycoside and cisplatin otoprotection.

Methionine (met) Safety Issues

- Met is a micronutrient and thus not alien to the human system
- Methionine comprises 26 mg/g high quality protein in the diet
- Has been used in human studies with no side effects (Kaji et al 1987, Kies et al 1975, Stegink et al 1986)

Met: Safety Issues (cont)

- World Health Organization lists methionine as essential drug for treating acetominophen overdose (2.5 gm doses at 4 hr intervals for a total of 10 gm over 12 hours)
- Monteagudo 1986 methionine “remarkably free of side effects” including nausea and vomiting
- DiRocco et al 1998 20gm/day safe for adults even for chronic administration

Advantage of D-met

- Although L-met would be safe even at high levels, D-met is even safer
- D-met has no apparent toxicity unless converted to the L isomer at high levels
- Toxicity can occur secondary to high dose L met or racemate in the presence of a very low protein diet (animal studies)

Advantage of D Met

- In humans, 60-70% of D-met is excreted without conversion (Printen et al 1979, Baker 1994)
- In humans D-met results in higher plasma levels than L-met
- D-met has longer half life and enhanced bioavailability (Borg and Walstrom 1989)

Over the counter (OTC) Methionine

- For decades OTC oral Met has been used to reduce urinary odor and associated dermatitis
- Recommended dosing is 200-400 mg orally 3-4 times per day

Kluge, Meech,and Campbell

- Effect of D-met on GSH/ GSSG with various duration noise exposures

Design-Details

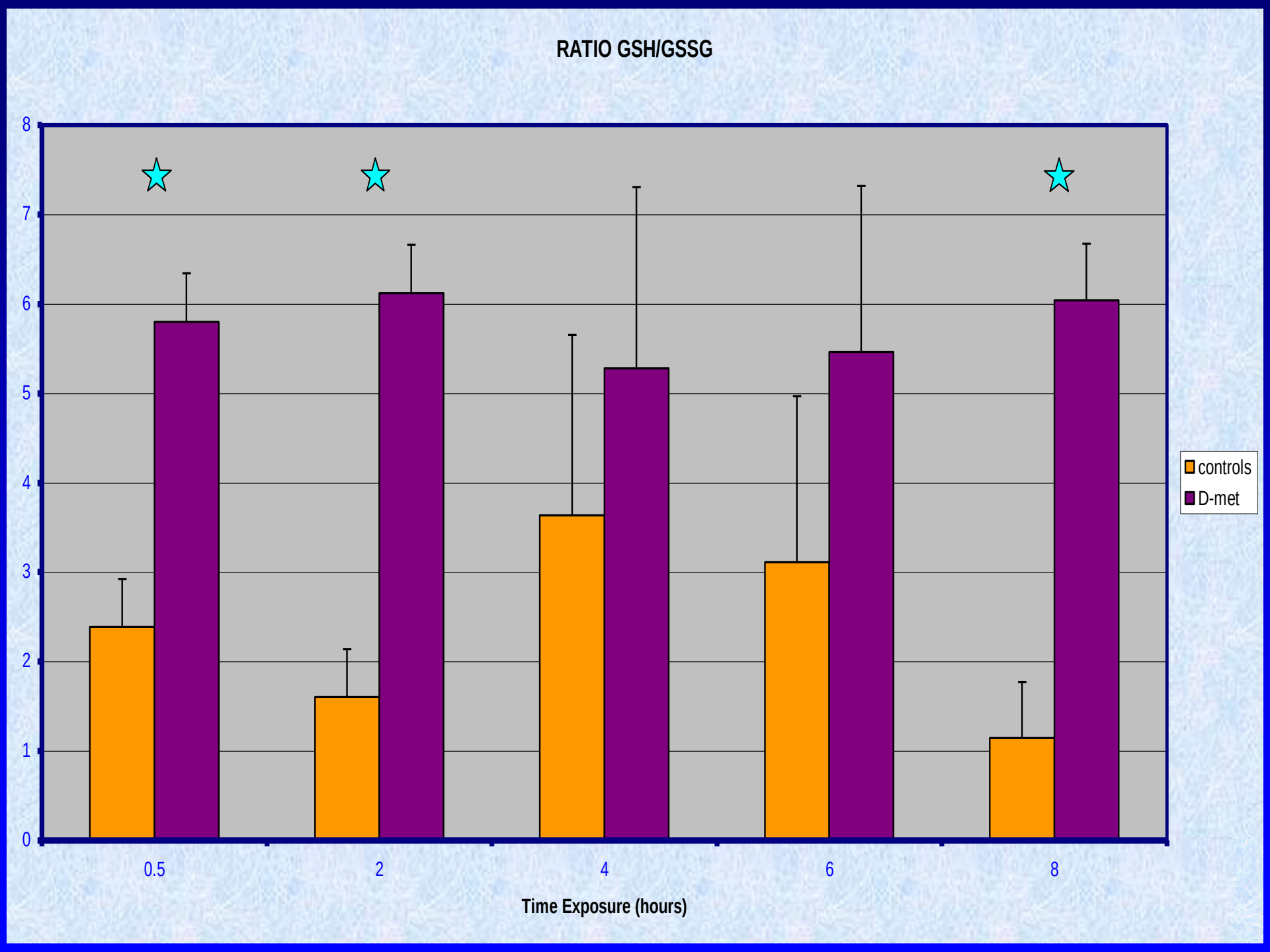
- 36 total animals
- 18 animals had intraperitoneal (IP) D-methionine injections (Experimental group)
- 18 animals had IP saline injections (Control group)
- Injections were twice-a-day for two days prior to exposure and on the morning of the exposure (total five injections per animal)
- The concentration of D-methionine was 200mg/kg

Noise Exposure

- The noise exposure was 105 dB SPL 4kHz NB noise
- There were three chinchillas in each noise exposure group
- The noise durations were:
  - None (15 min in booth with no sound on)
  - 30 min
  - 2 hr
  - 4hr
  - 6hr
  - 8hr

GSH, GSSG and Protein Determinations

- GSH and GSSG were determined by HPLC analysis
- Protein concentration was determined by spectrophotometry
- GSH and GSSG levels were standardized to the protein concentration in each sample


RATIO GSH/GSSG
8
7
6
5
4
3
2
1
0
0.5
2
4
6
8
Time Exposure (hours)
controls
D-met

Next step?

- Clinical trials to compare efficacy and side effects for cisplatin, carboplatin, aminoglycoside and noise otoprotection and for radiation induced oral mucositis
- More work on mechanisms
- Hopefully more than one agent will be FDA approved for all of these applications in the not too distant future.

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Discussion and Questions