

The Penn State McNair Journal

Summer 2005, Volume 12

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WELCOME

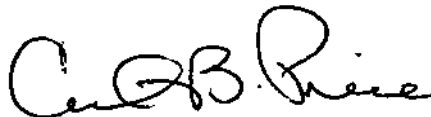
Since 1991, the Penn State McNair Scholars Program has enriched the lives of students at both Penn State and Virginia State University, our partner in the McNair collaboration. The McNair Program holds a very special place in our lives, as well as in the lives of the faculty and staff who work with our students. This publication celebrates their achievements and we offer it to our readers with pride and pleasure.

This is the twelfth issue of the Penn State McNair Journal. We congratulate the 2005 Penn State McNair Scholars and their faculty research advisors! This journal presents the research conducted in the summer of 2005 by undergraduate students from Penn State and Virginia State University who are enrolled in the Penn State McNair Scholars Program. The articles within this journal represent many long hours of mutual satisfying work by the Scholars and their professors. The results of their research are published here and have also been presented at various research conferences around the country. We are especially proud to see how these students have grown as researchers and scholars. The hard work, dedication, and persistence required in producing new knowledge through research is most evident in these articles. We very much appreciate the guidance, expertise, caring and patience of our fine group of Penn State faculty research advisors.

We are also fortunate to have the support and encouragement of many faculty and staff members who have worked with our students as social mentors or who have presented workshops and seminars on the many aspects of graduate and faculty life. You give the most precious of gifts to our students – your time in volunteering to support, encourage and nurture our Scholars' hopes and dreams.



Teresa Tassotti
Project Director



Curtis Price
Academic Coordinator

TRIO PROGRAMS ON THE NATIONAL LEVEL

Since their establishment in the mid-sixties as part of Lyndon Johnson's War on Poverty Program, TRIO Programs have attempted to provide educational opportunity and make dreams come true for those who have traditionally not been a part of the educational mainstream of American society. The TRIO programs are funded under Title IV of the Higher Education Act of 1965. While student financial aid programs help students overcome financial barriers to higher education, TRIO programs help students overcome class, social and cultural barriers to higher education. There are eight TRIO programs, which include the original three – Upward Bound, Talent Search and Student Support Services. The additional programs are Educational Opportunity Centers, Upward Bound Math & Science Centers, the Ronald E. McNair Post-Baccalaureate Achievement Program, a dissemination program, and a training program for TRIO staff. McNair programs are located at 180 institutions across the United States and Puerto Rico. The McNair Program is designed to prepare participants for doctoral studies through involvement in research and other scholarly activities.

TRIO PROGRAMS AT PENN STATE

The TRIO Programs at Penn State comprise six of the nine TRIO programs. There are two Educational Opportunity Centers, one in Philadelphia and the other in Pittsburgh; Ronald E. McNair Program; Student Support Services Program; two Talent Search Programs serving western Pennsylvania and York, Upward Bound; and Upward Bound Math & Science. These programs annually serve more than 5,000 students, from 6th graders through adults, with clear potential for academic success. The programs operate both at University Park and in communities across the state, often linking with middle schools, high schools, and community agencies. The programs focus on helping students overcome economic, social, and class barriers so that they can pursue education beyond high school.

MCNAIR SCHOLARS PROGRAM AT PENN STATE

Designed for low-income and first-generation college students, and students from groups underrepresented in graduate education, the McNair Scholars Program at Penn State encourages talented undergraduates from both Penn State and Virginia State University to pursue the doctoral degree. The program works closely with these participants through their undergraduate career, encourages their entrance into graduate programs, and tracks their progress to successful completion of advanced degrees.

The goal of the McNair Program is to increase graduate degree attainment of students from the above-mentioned underrepresented segments of society. McNair Scholars are presented with opportunities to study and do research in the University's state-of-the-art facilities in order to hone those skills required for success in doctoral education. Through both academic year and summer program components, McNair Scholars are required to complete a series of steps that lead to their application and enrollment in a graduate program of their choice.

Since 1991, the McNair Scholars Program at Penn State has helped 130 students earn their baccalaureate degrees. Of these graduates, 82 or 62 percent have gone on to graduate school at institutions across the country. And within this group, 17 or 21 percent have earned their doctoral or professional degrees and another 40 or 49 percent have earned their master's degrees. Currently, there are 38 or 29 percent of alumni who are enrolled in their graduate programs, some have earned their master's and are now pursuing their doctorates. Among the institutions McNair alumni have attended or now attend are: Penn State, Harvard, University of California-Berkeley, University of California-Davis, Stanford, Howard, Cornell, UCLA, Boston University, Indiana University-Bloomington, University of North Carolina-Chapel Hill, University of Maryland-College Park, John Hopkins University, University of Pennsylvania, Purdue, DePaul, and Ohio State, to name just a few.

Summer 2005 McNair Scholars and Program Staff



Standing (left to right): Curtis Price (Academic Coordinator), Teresa Tassotti (Program Director) Tamara Fleming (Summer Program Assistant), Judy Banker (Staff Assistant)

Sitting (left to right): Juliet Iwelumor, Syleena Guilford, Oneximo Gonzalez, Marie Krouse, Mike Lee, Keresha Deanes, Johnese Bailey

ABOUT RONALD E. MCNAIR

Dr. Ronald Erwin McNair, the second African American to fly in space, was born on October 21, 1950, in Lake City, South Carolina. In 1971, he received a Bachelor of Science degree, magna cum laude, in physics from North Carolina A&T State University. He continued his education at the Massachusetts Institute of Technology (MIT) where, in 1976, he earned his Ph.D. in physics.

While at MIT, McNair performed some of the earliest development of chemical and high-pressure CO lasers. He went on to study laser physics at E'cole D'ete Theorique de Physique in Les Houches, France. He was well published and nationally known for his work in the field of laser physics through the Hughes Laboratory.

In 1978, McNair realized his dream of becoming an astronaut when he was selected from a pool of several thousand applicants to be included in the first class of thirty-five applicants for the space shuttle program. Ronald McNair and six other astronauts died on January 28, 1986 when the space shuttle *Challenger* exploded after launching from the Kennedy Space Center in Florida.

McNair was an accomplished saxophonist; held a sixth-degree, black belt in karate; and was the recipient of three honorary doctorates and a score of fellowships and commendations. He was married to the former Cheryl Moore and is the father of two children, Reginald Ervin and Joy Cheray. After his death, Congress approved funding to honor the memory of McNair by establishing the Ronald E. McNair Post-Baccalaureate Achievement Program, which became the sixth program funded under the TRIO Programs umbrella.

“Historians, who will write about McNair, the man, will discover that there was much more to him than his scholastics achievements. Friends who knew him, say he walked humbly and never boasted about his achievements. They say his commitments were to God, his family and to the youths he encouraged to succeed.”
(Ebony, May 1986)

SPECIAL ACKNOWLEDGEMENTS

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McNair Alumni on the Move

We congratulate our recent graduates and are very proud of their accomplishments. We also extend congratulations to those Penn State McNair alumni who have earned their graduate degrees as well as those alumni currently enrolled in graduate studies.

At the graduate level...

Karla (James) Anderson (VSU 1999)	M.S., Central Michigan University
Michael Benitez (PSU 2001)	M.Ed., Penn State University
Jose Buitrago (PSU 1995)	M.L.A., Harvard University
Sherese Burnham (PSU 1999)	M.S., University of Central Florida
Aaron Brundage (PSU 1995)	M.S., Penn State University
	Ph.D., Purdue University
Sofia Cerda-Gonzalez (PSU 1999)	D.V.M., Cornell University
Debbie Charles (PSU 2001)	M.S., University of Maryland-College Park
Catherine Crawford (PSU 1996)	M.Ed., Central Michigan University
Natasha Deer (PSU 1995)	M.A., Florida State University
Alicia DeFrancesco (PSU 1997)	M.B.A., Babson College
Lurie Daniels (PSU 2000)	J.D., New York University
Eve Dunbar (PSU 1998)	Ph.D., University of Texas-Austin
Carol Elias (VSU 1997)	M.Ed., Virginia State University
Natasha Faison (PSU 1999)	M.S., Penn State University
Antoinette Gomez (PSU 1994)	M.S., Clark-Atlanta University
Cristina Gonzalez (PSU 1999)	M.D., Albert Einstein Medical School
Angela Hess (PSU 1998)	Ph.D., University of Iowa
Priscilla Hockin-Brown (PSU 1996)	M.S., Michigan State University
	Ph.D., Rutgers University
Marissa (Graby) Hoover (PSU 2000)	M.S., Temple University
Meng He (PSU 2002)	M.A., American University
Jeffrey Himes (PSU 1997)	M.S., West Virginia University
Alisa Howze (PSU 1994)	Ph.D., Texas A&M University
Andrea Jones (VSU 1998)	M.P.A., Virginia State University
Michelle Jones (PSU 1996)	Ph.D., Penn State University
Leshawn Kee (VSU 1998)	M.A., Regents University
Haroon Kharem (PSU 1996)	Ph.D., Penn State University
Carrie (Hippchen) Kuhn (PSU 2001)	M.A., Stanford University
LaShawne (Long) Miles (PSU 2001)	M.Ed., Xavier University
Charmayne Maddox (PSU 2004)	M.Ed., Penn State University
Debra Marks (VSU 1996)	M.S., University of Virginia
Robert Miller (PSU 1999)	Ph.D., University of Kentucky
Bethany Molnar (PSU 1998)	M.S., Northeastern University
Nicole Morbillo (PSU 1998)	Ph.D., New York University
Ndidi Moses (PSU 2000)	M.A., Penn State University,
	J.D., University of Connecticut
Robert Osmanski (PSU 1995)	M.S., Penn State University
Caryn Rodgers (PSU 2000)	Ph.D., St. John's University
Lilliam Santiago-Quinones (PSU 1998)	M.Ed., Bowling Green State University
Thomas Shields (PSU 1998)	M.A., Penn State University
Christie Sidora (PSU 2000)	M.A., Duquesne University
Melik Spain (VSU 1996)	M.S., Virginia Tech University

Anthony Spencer (PSU 1999)	Ph.D., Northwestern University
Shawyntee Vertilus (PSU 1998)	M.P.H./M.D., New York Medical College
Romon Williams (VSU 1995)	M.S., Wake Forest University
Wendy Williamson (PSU 1995)	M.B.A., Penn State University
Kenya Wright (VSU 1997)	M.S., North Carolina State University
Laurian Bowles (PSU 1999)	M.A., University of London, now pursuing Ph.D. at Temple University
Felecia Evans (PSU 2002)	M.S., Texas Tech University, now pursuing Ph.D. at Texas Tech
Janet Harris (PSU 1996)	M.S., Duquesne University, now pursuing Ph.D. at Duquesne
Rashid Njai (PSU 2000)	M.P.H., University of Michigan, now pursuing Ph.D. at University of Michigan
Mark Palumbo (PSU 2000)	M.S., Wright State University, now pursuing Ph.D. at Wright State
Zakia Posey (PSU 1999)	M.A., Michigan State University, now pursuing Ph.D. at Michigan State
Patrice White (VSU 2002)	M.S., University of Maryland-College Park, now pursuing Ph.D. at University of Maryland

At the undergraduate level...

Luis Agosto (PSU) May 2005
Hae Chung (PSU) May 2005
Andra Colbert (VSU) May 2005
Trinaty Crosby (PSU) May 2005
Kathy Goodson (VSU) May 2005
Marie Gutierrez (PSU) May 2005
Paula Henderson (PSU) May 2005
Jamal O'Garro (PSU) December 2004
Hui Ou (PSU) May 2005
Tiffany Polanco (PSU) December 2004
Jon Michael Tucker (PSU) December 2004

On to graduate school in Fall 2005...

Luis Agosto (PSU 2005) is pursuing graduate studies in Immunology at the University of Pennsylvania.
Trinaty Crosby (PSU 2005) is pursuing graduate studies in Social Work at Howard University.
Kathy Goodson (VSU 2005) is pursuing graduate studies in Biochemistry at the University of Maryland-College Park.
Marie Gutierrez (PSU 2005) is pursuing graduate studies in Middle Eastern Studies at the University of California-Berkeley.
Hui Ou (PSU 2005) is pursuing graduate studies in Industrial Engineering at Cornell University.
Tiffany Polanco (PSU 2004) is pursuing graduate studies in Animal Biosciences at Rutgers University.

In graduate school as of Fall 2005...

Juan Abreu (PSU 2002)
Taimarie Adams (PSU 2003)
Heneryatta Ballah (PSU 2004)
Omotayo Banjo (PSU 2004)
Jorge Delgado (PSU 2004)
Max Fontus (PSU 1999)
Tiana Garrett (VSU 2001)
Michael Godeny (PSU 2002)
Sherie Graham (PSU 2002)
Dustin Holloway (PSU 2002)
Mimi (Abel) Hughes (PSU 2002)

Lourdes Marcano (PSU 1995)
Leanna Mellott (PSU 2000)
Angel Miles (PSU 2003)

Edward Mills (VSU 2003)
Kizzy (Frey) Nicholas (PSU 2000)
Tracie Parker (VSU 2003)
Franche Pierre-Robinson (VSU 2002)
Kristin Rauch (PSU 2004)
Cavin Robinson (PSU 2002)
Luisa Soaterna (VSU 2004)
Kashra Taliaferro (PSU 2003)
Selima Taylor (PSU 2004)
Anthony Paul Trace (PSU 2004)

Kahlil Williams (PSU 2001)

Rutgers University (Law/Criminal Justice)
Harvard University (Law/Social Work)
Ohio State University (Women Studies)
Penn State University (Media Studies)
Purdue University (Aeronautical Engineering)
Indiana University-Bloomington (Chemistry)
University of North Carolina-Chapel Hill (Chemistry)
University of Florida (Physiology)
University of Michigan (Public Health)
Boston University (Molecular Biology)
University of Southern California (Atmospheric Sciences)
University of Tennessee (Business Administration)
Ohio State University (Sociology)
University of Maryland-College Park (Women's Studies)
University of Illinois at Urbana-Champaign (History)
Penn State University (Education)
Ohio State University (Music Education)
University of Illinois-Chicago (Education)
University of California-Davis (Anthropology)
DePaul University (Philosophy)
John Hopkins University (Biophysics)
University of Maryland-College Park (Education)
New York University (Health Science Administration)
University of Virginia (Medicine/Biochemistry & Molecular Genetics)
University of Pennsylvania (Political Science)

Does the CX₂CX₂C Motif Coordinate the [4Fe-4S] Cluster of NadA

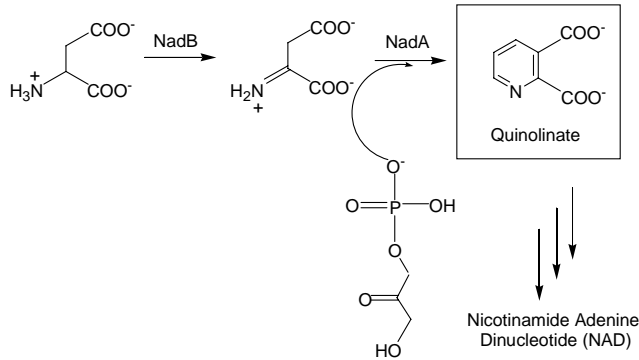
Johnese E. Bailey*, McNair Scholar, Virginia State University
Faculty Adviser: **Squire J. Booker, PhD.**, Associate Professor of Biochemistry and
Molecular Biology

Robert Cicchillo Kyung- Hoon Lee, Jeff Stromberg, Loretta Tu,
Department of Biochemistry and Molecular Biology
Eberly College of Science
Penn State University

Nicotinamide adenine dinucleotide (NAD) is a vital enzymatic cofactor and is present in all living organisms. ¹ NAD functions as a hydride acceptor resulting in the formation of its reduced form NADH, which is a hydride donor in redox reactions. In *Escherichia coli* (*E. coli*) and most other prokaryotes, NAD is produced from quinolinic acid, derived from the action of two enzymes, L-aspartate oxidase (NadB) and quinolinate synthetase (NadA) (Scheme 1). Recently, the Booker laboratory has shown that NadA harbors a [4Fe-4S] cluster. NadA contains nine cysteine residues, three of which lie in a motif (CXXCXXC) that is characteristic of cysteines that ligate iron-sulfur (Fe-S) clusters. Through expression of the *nadA* gene from *E. coli*, and purification and characterization of its gene product, lab the Booker lab intends to determine whether or not the cysteines in this motif coordinate the Fe-S cluster. Purification of the NadA protein was performed anaerobically due to the oxygen sensitivity of the cluster. Five variant NadA proteins have been generated, each of which contains a cysteine to serine substitution. With the removal of each of the cysteines, it is expected that formation of the Fe-S cluster will be impeded, resulting in an inactive NadA protein. The variant proteins will then be characterized by iron analysis and UV-Vis spectroscopy.

Introduction

Nicotinamide adenine dinucleotide (NAD) is a vital enzymatic cofactor that is present in all living organisms. NAD functions as a hydride acceptor resulting in the formation of its reduced form NADH, which is a hydride donor in redox reactions. Quinolinic acid, a precursor of NAD, is synthesized in *E. coli* by condensation of L-aspartate and dihydroxyacetone phosphate (DHAP) in an anaerobic pathway ^{2,3} (Scheme 1). This reaction requires two essential enzymes, L-aspartate oxidase (NadB) and quinolinate synthetase (NadA). The *nadA* gene has been cloned and sequenced, however little work has been published describing the mechanism of NadA because of its instability. The NadA protein harbors an oxygen sensitive iron-sulfur (4Fe-4S) cluster, which has hampered many attempts to purify it. ⁴



Scheme 1 The biosynthetic pathway of NAD in *E. coli*.

Three cysteines are believed to coordinate the Fe-S cluster, and in *E. coli* it has been suggested that the cysteines lie in a CX₂CX₂C motif, which is known to involve iron-sulfur cluster coordination in other iron-sulfur proteins⁵. Research done in the Booker lab has verified that a [4Fe-4S] cluster is present in NadA of *E. coli*³; however, the cysteines that coordinate the cluster have not been established. To assess whether or not cysteines in the CX₂CX₂C motif coordinate the cluster, we initially carried out an exhaustive sequence alignment of the NadA protein from over 150 species. We found that only three cysteines were conserved throughout all species, only one of which lies in the CX₂CX₂C motif (Scheme 1). To verify that the conserved cysteines were those that ligand the cluster, we generated Cys to Ser variants of each of the cysteines, and isolated the corresponding proteins to investigate their role in the ligation of the [4Fe-4S] cluster.

Escherichia	MSVMFDPDTAIYFPFPKPTPLSIDEKAYYREKIKRLLKERNAMVMVAHYTDP EIQQLAAE	60
Shigella	MSVMFDPDTAIYFPFPKPTPLSIDEKAYYREKIKRLLKERNAMVMVAHYTDP EIQQLAAE	60
Yersinia	MSEIFDVNAAIYFPFPARVPLDTNEKAFYREKIKTLLKQRDAVLVAHYTDP EIQALAAE	60
Erwinia	MNILFDSNETIYFPFPKPRPLSVDKQHYRSRIKTLRERNAVMVAHYTDP EIQALAAE	60
	*. : ** : : ***** : * ** . : * . ** . : ** ** : : * : * : *****	
Escherichia	TGGCISDSLEMARFGAKHPASTLLVAGVRFMGETAKILSPEKTIILMPTLQAECSLDLGC	120
Shigella	TGGCISDSLEMARFGAKHPASTLLVAGVRFMGETAKILSPEKTIILMPTLQVECSLDLGC	120
Yersinia	TGGCVADSLEMARFGNHPASTLLVAGVRFMGETAKILNPEKKVLMPTLNACSLDLGC	120
Erwinia	TGGCVADSLEMARFGSTHSASTLLVAGVRFMGETAKILNPEKTIILMPTLEACSLDLGC	120
	***** : ***** . * . ***** : ***** . * . * . : ***** : *****	
Escherichia	VEEFNAFCDAHDPDRTVVVYANTSAAVKARADWVVTSSIAVELIDHLDLGEKI IWAPDKH	180
Shigella	VEEFNAFYDAHDPDRTVVVYANTSAAVKARADWVVTSSIAVELIDHLDLGEKI IWAPDKH	180
Yersinia	VDEFTAFCDSDHPDRTVVVYANTSAAVKAKADWVVTSSIAVELIEHLDLGEKI IWAPDRH	180
Erwinia	IDAFSRFCDAHDPDRTVVVYANTSAAVKARADWVVTSSIAVELIEHLDLGEKI IWAPDRH	180
	:: * . * * : ***** : ***** : ***** : ***** : *	
Escherichia	LGRYVQKQTGGDILCWQGACIVHDEFKQTALTRLQEEYPDAAILVHPESPQAIIVDMADAV	240
Shigella	LGRYVQKQTGGDILCWQGACIVHDEFKQTALTRLQEEYPDAAILVHPESPQAIIVDMADAV	240
Yersinia	LGSYVQKQSGADVLCWQGACIVHDEFKQTALARMKALYPDAAVLVHPESPQAVVDMADAV	240
Erwinia	LGSYVQKQTGADVLCWQGACIVHDEFKQTALQRMKILYPDAAILVHPESPQSVVEMADAV	240
	** ***** : * . * : ***** : * : : ***** : ***** : : * : *****	
Escherichia	GSTSQLIAAAKTLPHQRLIVATDRGIFYKMQQAVDPKELLEAPTAGEGATCRSCAHC	300
Shigella	GSTSQLIAAAKALPHQRLIVATDRGIFYKMQQAVDPKELLEAPTAGEGATCRSCAHC	300
Yersinia	GSTSQLIQAAKTLPQKTLIVATDRGIFYKMQQACPKELFEAPTAGEGATCRSCAHC	300
Erwinia	GSTSQLIQAAKTLPQRELIVATDRGIFYKMQQACPEKTLLEAPTAGEGATCRSCAHC	300
	***** * : * : * : ***** : ***** * : * * : ***** : *****	
Escherichia	AMNGLQAI AEALQEGSNHEVHVDERLRERALVPLNRMLDFAATLRG-----	347
Shigella	AMNDLQAI AEALQEGSNHEVHVDERLRERALVPLNRMLDFAATLRG-----	347
Yersinia	AMNGLRAIAEGLQGGVMHEIHVDEBLRQALIPLNRMDFANQLKLVKGN	353
Erwinia	AMNGL EAI ANGLEQGGHAHEIHVDAALREGALIPLNRMDF AASLKL RVKGN	353
	*** : ***** : ***** * : * : ***** : ***** *	

Figure 1. Primary sequence alignment of the NadA protein from *Escherichia coli*, *Shigella flexneri*, *Yersinia pestis*, *Erwinia carotovora*. * = conserved amino acid residues

The highlighted residues denote the conserved cysteines that were changed to serine.

Materials and Methods

Cloning NadA gene from E. coli

Loretta Tu, a technician in the Booker lab, generated the C113S, C200S, C291S, C294S, and C297S substitutions in the *nadA* gene. The genes were cloned into a pET-28a vector such that the gene products would contain a hexahistidine appendage at the N-terminus to facilitate purification by immobilized metal affinity chromatography (IMAC).

Growth and expression of NadA

The *C*₂₉₇ *nadA* mutant strain was streaked onto a Luria Bertani (LB) plate containing 50 $\mu\text{g mL}^{-1}$ of kanamycin (kan) and 100 $\mu\text{g mL}^{-1}$ of ampicillin (amp). The plate was then grown overnight at 37°C. A single colony from this plate was used to inoculate 200 mL of LB containing 50 $\mu\text{g mL}^{-1}$ of kan and 100 $\mu\text{g mL}^{-1}$ of amp. The starter culture was grown at 37°C for 10-15 hrs (until turbid). This culture was used to inoculate 4 flasks, each containing 4L of LB with the previously stated antibiotics at the same concentration. At an optical density of ~0.3, 200 mg of arabinose was added to each flask. At an optical density of ~0.6, the bacteria were induced with 200 μM of IPTG. Expression of the *nadA* gene was allowed to continue for 4 hrs at 37 °C. The cultures were then placed in an ice bath to cool. The cells were then harvested by centrifugation at 10,000 x g for 10 minutes. The cell paste was then frozen in liquid nitrogen and stored in a Cryo-dewar. Typical growths yielded 30-40g of frozen cell paste.

Anaerobic Purification of NadA

Cells (30g) harboring the *nadA* *C*₂₉₇S variant was thawed in 80 mL of Lysis (50 mM Hepes, 0.3 M KCl, 20 mM Imidazole, 10 mM BME) inside of a Coy anaerobic chamber. The cell paste was stirred at room temperature, and once the cells were thawed, lysozyme was added to a final concentration of 1 mg mL⁻¹ and the solution was allowed to stir for an additional 30 min. Cells were incubated on ice to bring the temperature to <8 °C, after which they were disrupted by sonication (4 x 1 min at a setting of 7) using a Sonicator Ultrasonic Processor (Fisher Scientific; Pittsburgh, PA). The solution was then centrifuged at 50,000-x g for 1 hour to remove cellular debris. The supernatant was loaded onto a nickel-nitriloacetic acid (Ni-NTA) agarose column equilibrated in cold Lysis Buffer. The column was then washed with 70 mL of Wash Buffer (50 mM Hepes, 0.3 M KCl, 40 mM Imidazole, 10mM BME, 20% Glycerol). The protein was eluted from the column using Elution Buffer (50 mM Hepes, 0.3 M KCl, 250 mM Imidazole, 10mM BME, 20% Glycerol). Fractions containing protein (based on a brown color) were collected into test tubes and placed on ice. The protein was then concentrated to ~1 mL using an Amicon ultrafiltration unit with a 10,000 molecular weight membrane, and subjected to gel filtration using a G-25 column pre-equilibrated in 50 mM EPPS pH 8, 100 mM KCl, 20% glycerol, 10 mM DTT. The concentrated protein was aliquoted (200 μL) and stored in a Cryo-cooler

Protein concentration analysis

Protein concentration was determined using the method of Bradford⁶ using reagent purchased from Pierce (Rockford, IL) and bovine serum albumin (BSA) as the standard. A color change of brown to blue indicated the presence of the protein.

UV-Vis spectroscopy

UV-Vis spectra were recorded using a Cary 50 (Varian; Walnut Creek, CA) in combination with the associated WinUV software package.

SDS-PAGE analysis of the protein

The purity of the protein was assessed using SDS-PAGE as described by Laemmli.⁷ The molecular mass of the protein was determined using molecular mass standards obtained from Amersham (Piscataway, NJ).

Iron Analysis

The iron content in the purified NadA proteins was determined using the method of Beinert.⁸ The concentration of iron was correlated to that of the protein to determine the number of irons present per polypeptide.

Results

Purification of the NadA Protein

The NadA C₁₁₃S, C₂₀₀S, C₂₉₁S, C₂₉₄S, and C₂₉₇S variants containing a hexahistidine tag at the N-terminus were anaerobically purified. The purified proteins were brown, indicative of a Fe-S cluster. After purification, the C₂₉₁S and C₂₉₄S variants displayed a deep brown color, while the color of the C₂₀₀S, C₁₁₃S, and C₂₉₇S variants was not nearly as intense. This difference suggests that cysteines 291 and 294 do not ligate the cluster whereas cysteines 200, 113, and 297 probably serve as ligands.

Spectroscopic Analysis

Spectroscopic studies were performed to determine whether the brown color of the proteins was a result of the presence of a [4Fe-4S] cluster. The UV-visible spectrum of the NadA variants is displayed in Figure 2. As can be observed, each of the spectra shows an intense peak at 276 nm and a broader but weaker feature at 420 nm. The peak at 276 nm correlates with the protein component of each of the variants, while the feature at 420 nm correlates with the [4Fe-4S] cluster component. For the C₂₉₁S and C₂₉₄S variants the intensity of the peak at 420 nm was almost 25% of the peak at 276 nm, which implies that there is a substantial amount of 4Fe-4S cluster present in these proteins; however, this was not the case for the C₂₀₀S, C₁₁₃S, and C₂₉₇S variants (ratio was less than 10%). Therefore we concluded that C₂₉₁ and C₂₉₄ in the wild type protein do not ligate the cluster, whereas C₂₀₀, C₁₁₃, and C₂₉₇ may be essential for the ligation of [4Fe-4S] cluster.

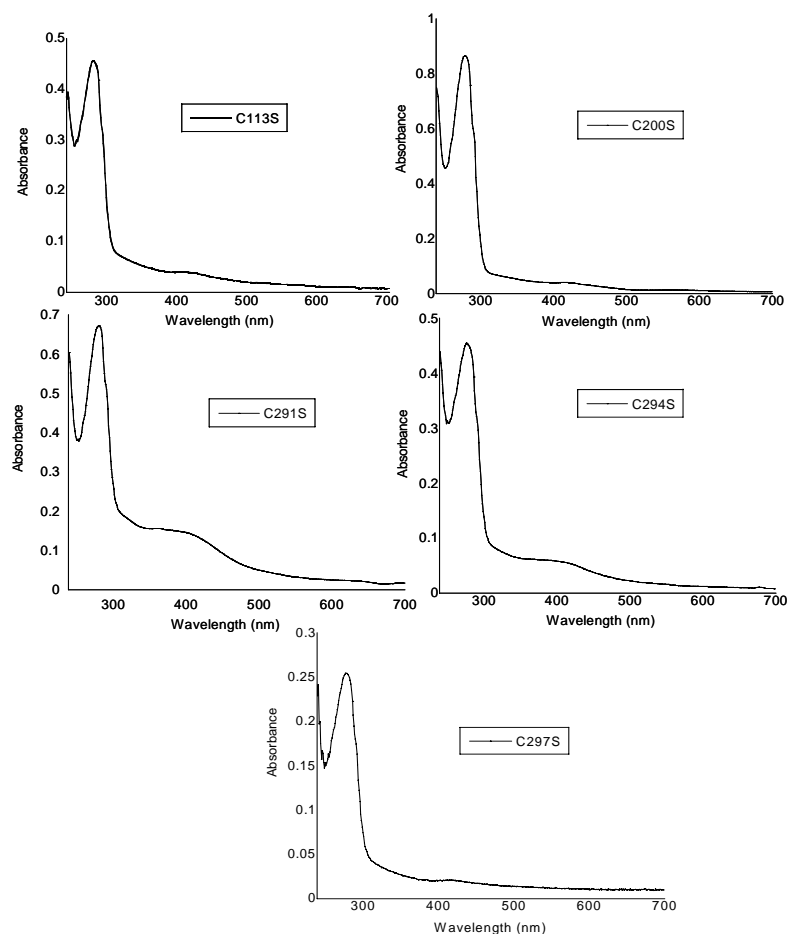


Figure 2. UV-Vis Spectra of the five variants of the NadA protein.

Further characterization of NadA was performed to determine the iron content using the method of Beinert. To determine the number of irons present in the protein, we compared the concentration of iron obtained to that of the protein as determined using the method of Bradford with BSA as the standard. For a [4Fe-4S] cluster it is expected that the protein should contain four irons for every polypeptide. Our results (Figure 3) indicate that the iron content was negligible for C₂₀₀S, C₁₁₃S, and C₂₉₇S variants. We did, however, observe ~2 irons per polypeptide for the C₂₉₄S variant and ~3 irons per polypeptide for the C₂₉₁S variant.

Enzyme	Fe/ Protein
C ₁₁₃ S	< 1
C ₂₀₀ S	< 1
C ₂₉₁ S	3.27
C ₂₉₄ S	1.73
C ₂₉₇ S	< 1

Figure 3. Fe Analysis of NadA protein

Conclusion

The results of this study indicate that not all of the cysteines within the motif presumed to ligate the [4Fe-4S] cluster (C₂₉₁XXC₂₉₄XXC₂₉₇) serve as ligands, but that two of the ligands derive from other regions of the NadA protein sequence. Based on spectroscopic analysis we observed that C₂₉₁ and C₂₉₄ had no involvement in coordinating the cluster, whereas C₁₁₃, C₂₀₀, and C₂₉₇ show potential as ligands to 4Fe-4S. To better confirm our preliminary findings, activity assays, sulfide analysis, electron paramagnetic resonance (EPR) spectroscopy, and Mössbauer spectroscopy will be performed on the protein.

Since the primary function of NAD is to carry out redox reactions in all living organisms its biosynthesis is crucial for cell viability. Both prokaryotes and eukaryotes biosynthesize NAD from quinolinic acid, but the pathway by which quinolinic acid produced is different between the organisms in these two kingdoms. This suggests that NadA might serve, as a potential target for the generation of antibacterial agents.⁹ Thus, understanding the mechanism by which NAD is produced in bacteria is vital.

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Analysis and Characterization of Stapedius Muscle Electromyograms Elicited by Cochlear Implants in Rats.

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Introduction

Cochlear implants (CI) are the most successful and popular neural prostheses used in our time. By means of electrical stimulation, this device is capable of restore partial to complete auditory sensation in patients suffering of sensorineural hearing loss. A microphone captures the sound which is converted into electrical pulses by a processor, and sent to an array of electrodes implanted in the cochlea. These pulses activate the auditory nerve and are ultimately perceived as sound by the brain.

After surgical implantation of a CI, a series of programming sessions have to be conducted in order to fit the device. This is a key step for optimal performance and is both challenging and time consuming. Usually, devices are fit based upon subjective behavioral tests and patient feedback to determine a range of stimulation in which the device will operate, often called the dynamic range. These methods, especially for setting the upper limit of stimulation or comfort level (C-level), are not very reliable and present a lot of difficulties with patients that are pre-lingually deaf and with young children that are not able to provide clear, objective judgments regarding their listening level. [1, 2]

Several objective electrophysiological measures have been proposed as an aid for the fitting of CI. [3] The electrically evoked auditory brainstem response (EABR) has been used to evaluate candidacy for cochlear implantation, and recently to assist in the mapping of CI. Whereas, the electrically evoked compound action potential (ECAP) (also called the Neural Response Telemetry (NRT) when using the Nucleus 24 device), has been used to confirm response to electrical stimulation and assisting with setting the programming levels.

Furthermore, the most common measure for the fitting of CI is the electrically evoked stapedius reflex (ESR), measured by monitoring the changes in the eardrum acoustic impedance. The correlation of this measurement with the upper limit of the dynamic range or comfort level (C-level) has been shown through various studies. [1,4,5] Although, the ESR is a good way of establishing the upper limit of stimulation, between 30-40% of cochlear implant patients have not shown any detectable impedance changes due to electrical stimulation. [3,8] The reasons for the absence of the reflex when measuring the impedance can be due to middle ear malformations, motion artifacts, and other reasons that in some cases cannot be determined. It does not mean necessarily that the reflex is absent, just that a better method to obtain it is needed. In addition, children can have a hard time remaining stationary or tolerating the probe that's used to measure the impedance change. [6, 7]

Using stapedius muscle electromyograms (SEMG) has been proposed as a promising, although invasive, method of recording the ESR more effectively. [8] Other important aspects for the use of this measurement still need to be examined however. In this study, we use an

animal model to investigate the relationship that the less invasive measurements, EABR and ECAP, have with the SEMG to test if independent information can be obtained from them. The effects of high rates of stimulation on the SEMG response are also examined. The results demonstrate the viability of using SEMG as an objective measurement to aid in the process of fitting CI.

Methods

Surgical Procedures

Four healthy Sprague-Dawley rats (300-500g) were anesthetized with a mixture of ketamine/xylazine/acepromazine (50:5:1 mg/kg) with supplemental anesthesia given to maintain areflexia during the entire surgical procedure. The physiological state of the animal was monitored by blood oxygen saturation and heart rate. The animals were placed on a heating blanket maintained at 37 degrees Celsius. All animal procedures followed NIH guidelines and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee's (IACUC).

The top of the skull was cleared and bone screws were placed to anchor a dental acrylic skullcap. One of the screws served as a ground for the EABR electrodes that were sutured to fascia of muscles near the ear canal. A nut was fixed in the acrylic skullcap for a head manipulator to be locked on it and allow the head to be fixed at an appropriate angle for surgical approach to the middle ear.

A post-auricular incision was made, and the tendon of the m.sternomastoideus muscle was identified and followed to the bulla. The tissue surrounding the bulla was removed, and an accessory hole was drilled until the cochlea, round window and stapedius were visualized. Sounds were made to visually confirm the contraction of the stapedius muscle. The stapedius electrodes were placed close to the stapedial cavity with the aid of a manipulator, and pushed to the muscle with forceps. A cochleostomy was made and the cochlear electrodes placed into the first turn of the cochlea. The bulla was repaired with carboxylate cement. The various recording electrodes were connected to a multi-channel programmable amplifier system (AMsystems model #2000).

Electrodes design.



Figure 1. Stapedius Electrodes from 50 μ m tungsten micro wires.

The SEMG electrodes were composed of 50 micrometer diameter insulated tungsten micro wires that were electrolytic sharpened at the tips. Two electrodes were slid together into polyimide tubing and fixed with epoxy to form a bipolar pair approximately 400 micrometers apart. The electrode tips were bent at a right angle to allow easier insertion into the stapedial cavity.

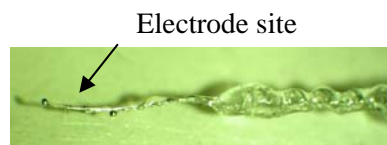


Figure 2. Cochlear Implant electrodes from 25 μ m Pt-Ir micro wires.

The cochlear implant electrodes were three or four channel hand fabricated ball electrode arrays. They were fabricated from 25 micrometers insulated Pt-Ir wire with ball ends made with a micro-torch. Three or four of these wires were threaded together, with a spacing of ~500 micrometer from ball to ball.

Stimulation and Electrophysiology Recordings

The cochlea was stimulated using biphasic pulse trains delivered through a Nucleus CI24M cochlear implant system (Cochlear Corporation). Stimulation was delivered either between two intra-cochlear electrodes (bipolar) or an intra-cochlear and an extra-cochlear reference (monopolar). Current levels varied up to a maximum of 1750mA. Pulse widths were 25-50 microseconds. The stimuli for EABR and ECAP responses were single biphasic current pulses presented at 10 Hz. Stimuli for obtaining growth functions of the SEMG were delivered in 250 milliseconds bursts separated by an inter-stimulus interval (ISI) of 2 seconds.

EABR and ECAP were filtered (0.3 Hz-20 kHz), amplified (20k gain) and sampled at 50 kHz. All results presented were obtained from stimulation delivered to the middle channel.

The stapedial EMG was recorded differentially from the bipolar SEMG electrodes. The SEMG was filtered (300 Hz-20 kHz), amplified (20k gain) and sampled at 50 kHz. The stimuli consisted of 10-20 presentations delivered in 250 milliseconds bursts with an ISI of 2 seconds. Pulse widths were 25-50 microseconds with frequencies of 250 Hz, 500 Hz and 1 kHz.

Data Analysis

ECAP response was obtained calculating the magnitude of the first wave from the EABR electrodes. Intracochlearly, the ECAP wave was the only one measured by the electrodes.

Growth functions were obtained by plotting the average magnitude of each response against the current level. When normalizing, each corresponding value was divided by the maximum magnitude in the series.

Artifact was eliminated in the SEMG recordings by a threshold algorithm that blanked out any magnitudes above it, the values were adjusted to take into account the “dead time”, leaving just the stapedius muscle response.

Results

Representative samples of the ECAP and EABR recordings from the cochlear and stapedius electrodes, and the EABR electrodes respectively, during single pulses stimulation are shown in Figure 3. Each plot represents the average response to 600 stimulus presentations (300 in one polarity and 300 in the opposite polarity to reduce artifact) superimposed at different current levels ranging from 170 to 1581uA. Threshold was observed around 170 and 255 uA, when a visible response was noted.

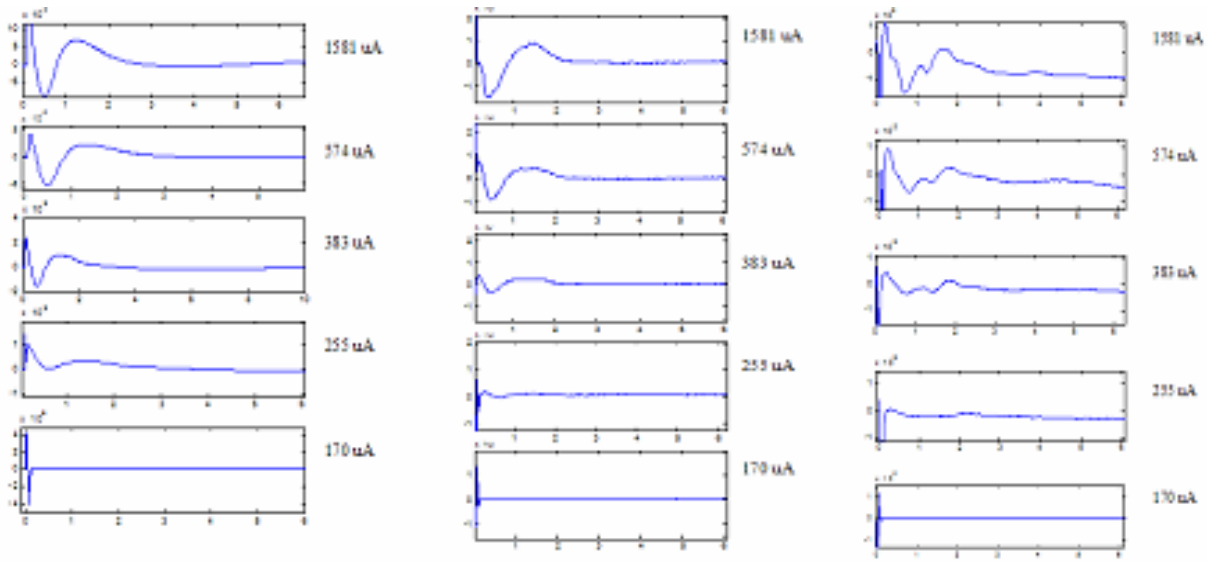


Figure 3. ECAP recorded from the cochlear electrodes (column 1), the stapedius electrodes (column 2), and the EABR electrodes (column 3).

The ECAP response obtained from three different places is shown in Figure 4. Using the stapedius electrodes to get the ECAP response has never been reported before, and represents an alternative function of these electrodes. The responses from the different methods varied in magnitude, but after normalizing each, as shown in Figure 5 (dividing by the greatest response), it is noticeable the similarity in morphology of these three techniques.

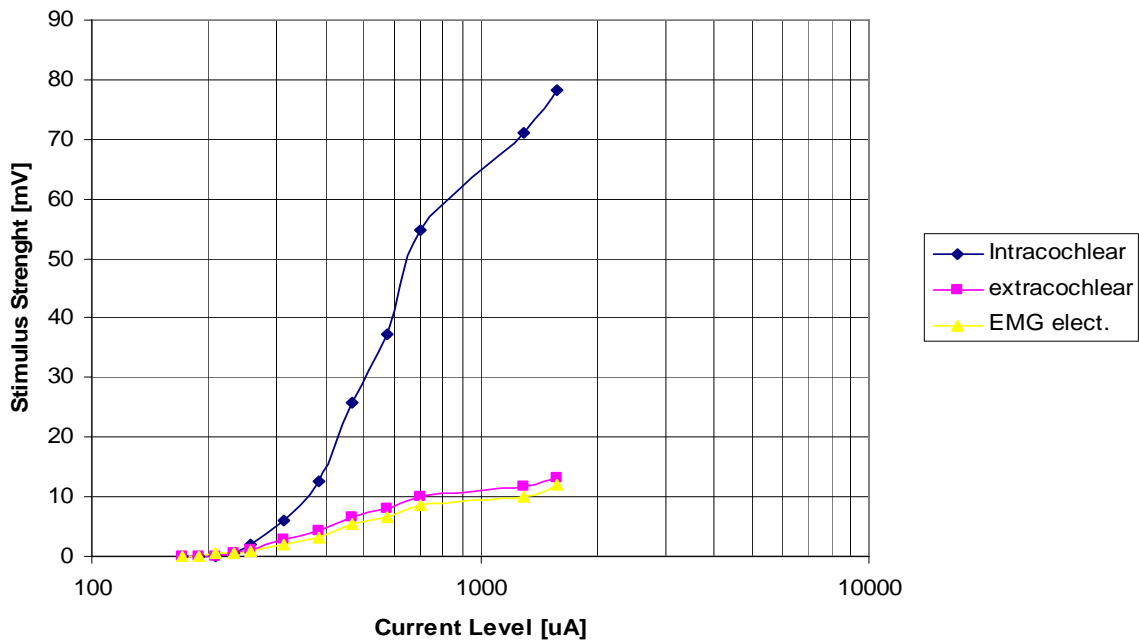


Figure 4. ECAP response growth from the cochlear, EABR and stapedius electrodes.

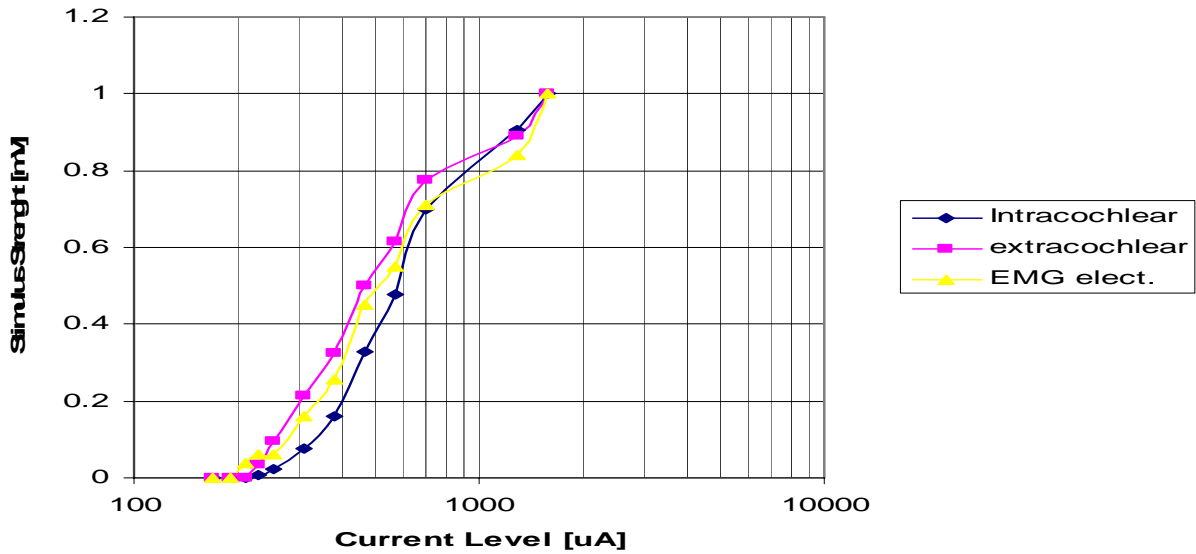


Figure 5. Normalized ECAP response growths from the cochlear, EABR and stapedius electrodes.

The SEMG response characteristics at different stimulus rates are shown in Figure 6. An evident increase in threshold is observed as rate increases. On Figure 7, response growths obtained from a previous animal at different rates of stimulation support our observations for the current study [8].

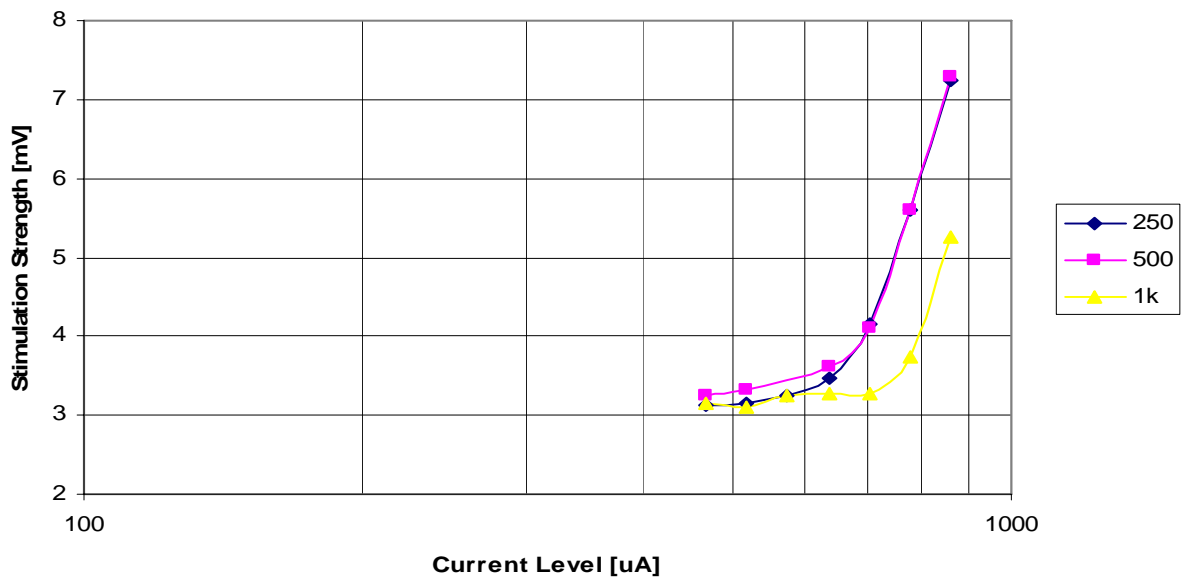


Figure 6. SEMG responses at 250 Hz, 500 Hz and 1 kHz.

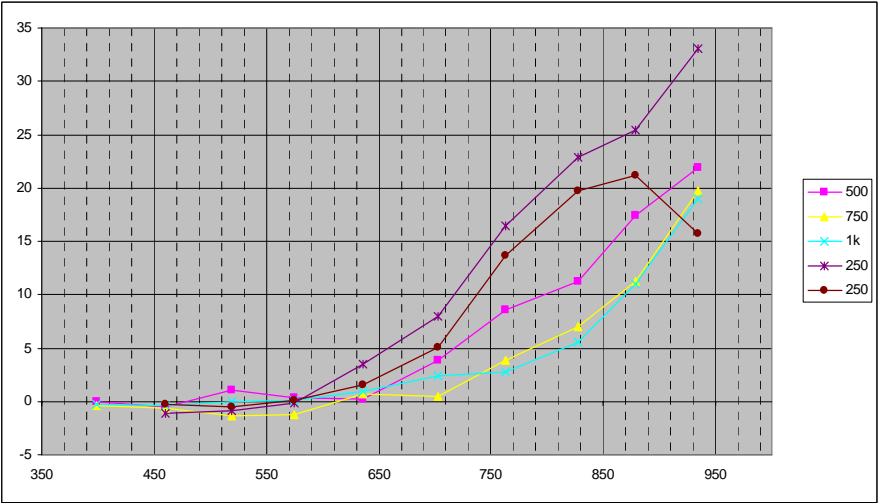


Figure 7. SEMG responses at 250 Hz, 500 Hz, 750 Hz and 1 kHz.

The ECAP thresholds were lower than the ESR threshold as would be expected (313 uA for the ECAP, 703 uA for the ESR, Figures 8 & 9). The amplitude between the ECAP growth function with that of the SEMG growth functions were also not consistent, suggesting that both signals could provide independent information about the level of stimulation. A similar trend was observed between the EABR growth function (threshold: 210 uA) and the SEMG growth functions (threshold: 390 uA) (Figure 10).

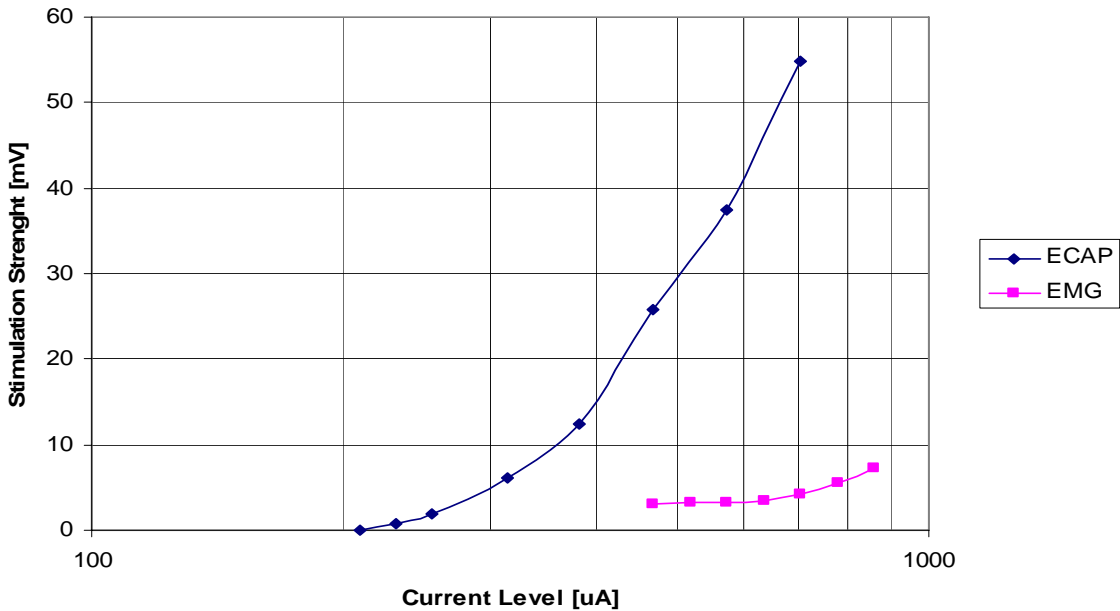


Figure 8. Comparisons between the ECAP growth functions and the SEMG growth functions. Threshold for the ECAP was around 313 uA and for the SEMG around 703 uA.

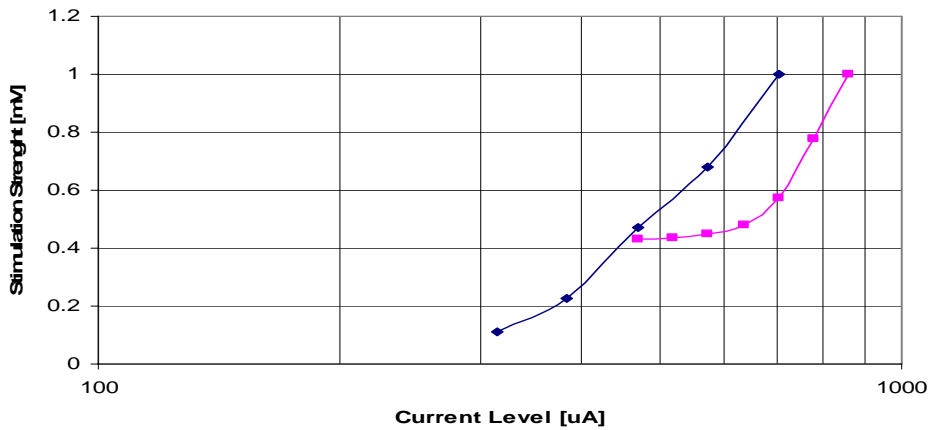


Figure 9. Comparison of the normalized growth functions of the ECAP response and the SEMG response.

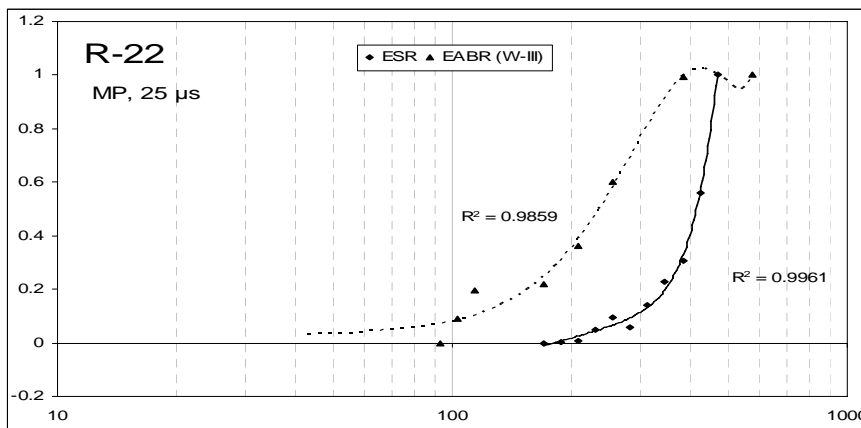


Figure 10. Comparison between the EABR growth function and the SEMG growth function.

Discussion and Conclusions

We have successfully explored the features of the stapedius muscle activation using electromyograms during high rates of stimulation with a cochlear implant and compared the response with other objective measurements. The threshold of the SEMG was observed to increase as the rate augmented. A new function of the stapedius electrodes to capture the ECAP response, when single pulses stimuli is used, was also encountered. The SEMG technique is a feasible alternative for characterizing the electrical stapedius reflex for cochlear implant fitting. In the future, further research to study the effects that deafness has on the response and to develop a chronic model will be done. New ways of dealing with artifact are yet to be explored. The SEMG signal could be someday incorporated into the cochlea implant system to provide an automatic gain control of the stimulating current.

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Effects of Acute Stress on Brachial Artery Blood flow Measured via Ultrasound

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Abstract: We conducted a randomized, controlled trial to examine the effects of acute stress on vascular endothelial function in 29 men and women with elevated or normal cholesterol. In previous studies, a significant reduction in endothelial function in the immediate post stress period was observed. It is possible that differences in blood flow after stress may explain the variability observed by past studies. The goal of this research was to examine post-stress changes in brachial artery blood flow and blood velocity and to test whether cholesterol status alters this hemodynamic response to stress. We found significant reductions in baseline blood flow volume and blood flow velocity at 45 and 90 min post-stress. In addition, the percent increase in volume and velocity caused by cuff deflation was higher at 90 min post-stress vs. the resting baseline. There were no significant differences in blood flow responses between subjects with high vs. low cholesterol.

Introduction

Cardiovascular disease

Cardiovascular disease is the leading cause of death in industrialized countries. According to the American Heart Association, in 2002 cardiovascular disease impacted 70.1 million adults (34.2% of the total population) and the mortality rate was 927.4 thousand. This disease is prevalent in Western cultures. Some of the risk factors that are seen in individuals who have this disease are high blood pressure, tobacco use, high blood cholesterol, physical inactivity, overweight, obesity, and diabetes mellitus (AHA 2005).

There have been many studies to help inform researchers and the general population about this disease, but one important, landmark study has documented the progression of the disease over a period of time, within the same individuals. The Framingham Heart Study was funded by the federal government in Framingham, Massachusetts to learn the circumstances under which this disease effects the general population. In 1948, researchers recruited 5,029 men and women between the ages of 30 and 62 and began a project, which would impact society for many years to come. This study recruited a second generation in 1971 that consisted of 5,124 of the original participants' adult children and their spouses. The third generation (the children of the offspring cohort) is currently being recruited and examined to seek further understanding of how genetic factors relate to cardiovascular disease (NHLBI, 2002). The participants of this study have been monitored very closely through cardiovascular examinations, hospital admissions information, death information, information from physicians and other sources outside the clinic (AHA, 2005). The objective of this study was to identify that common factors or characteristics that contribute to cardiovascular disease by following the development of the disease over a period of time. This study has better informed researchers and the general public about the etiology of cardiovascular disease (NHLBI, 2002).

Although there are many factors, which contribute to cardiovascular disease atherosclerosis, is one of the major causes. Atherosclerosis is a build up of plaque (deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances) within the inner lining of an artery wall (AHA, 2005). This disease begins in childhood and progresses into adulthood. Studies have shown that this disease is genetic but it also is prevalent in individuals with elevated levels of cholesterol and triglycerides in the blood, those with high blood pressure, and individuals exposed to tobacco smoke (AHA, 2005).

Cardiovascular disease also has put a strain on the U.S. economy. According to American Heart Association, the U.S. spent an estimated \$393.5 billion in 2005 on cardiovascular disease. These costs included direct costs (physicians, other professionals hospital, and nursing home services, medication, home health care and other medical supplies) and indirect costs (lost productivity resulting from morbidity and mortality). In contrast, the estimated cost of all cancers was half this amount (estimated at \$190 billion-direct and indirect costs) in the same time period (AHA 2005).

Stress and Cardiovascular Disease

Cardiovascular disease and stress have been researched to show if there is a relationship between the two factors. Some of the factors that initiate stress are job strain, personal problems, and social interaction but more research needs to be done to better understand the mechanisms through which stress promotes vascular disease. Researchers are unsure if stress is an independent risk factor for cardiovascular disease; but stress may have a direct correlation with other risk factors such as high blood pressure, high cholesterol levels, smoking, physical inactivity, and unhealthy diet (AHA 2002). Strike and Steptoe (2004) summarized several studies on the role of psychological factors in CVD and reported a correlation between chronic stress, low socioeconomic status, depression, and social isolation and development of coronary artery disease and suggested that these characteristics can be seen as CVD risk factors (Strike and Steptoe 2004). In addition, in the Kuopio Ischemic Heart Disease Study, 901 Eastern Finnish men from four age cohorts (age 42 to 60 years) were studied to understand the correlation of cardiovascular reactivity to mental stress and atherosclerotic risk. Researchers found that men with the largest cardiovascular responses during exposure to mental stress had more severe atherosclerosis. (Kamarck et al.,1997). Although studies have shown the correlation between the two, there are many other factors, which contribute to this disease, and researchers acknowledge that psychosocial factors may not directly cause CAD (Strike and Steptoe, 2004).

Stress and Endothelial Function

The endothelium layer of the vascular walls regulates the blood flow and helps prevent atherosclerosis. This layer also responds to changes in blood flow such as shear stress; where it secretes vasodilators (nitric oxide (NO), prostacyclin) and vasoconstrictors (endothelin-1, angiotensin II), which regulate the dilation and constriction of vessels (West, 2001). Some recent studies have suggested that endothelial function is altered by stress (Ghiadoni, et al, 2000). The following coronary risk factors are also associated with the impairment of endothelial function: smoking, elevated cholesterol, saturated fat, obesity, and diabetes. (AHA 2005). Endothelial function is also related to flow mediated dilation (FMD), which is a non-invasive measure for endothelial health. Brachial artery flow mediated dilation is the most commonly used measure of endothelial function. FMD is based on the understanding of how vascular smooth muscle responds to increases in blood flow (Sinoway et al, 1989). Blood vessels respond to their environment by dilating or constricting depending on the conditions they are under. The increase in blood flow causes shear stress on the endothelium, triggering NO release in healthy arteries, and brachial artery FMD measures how well the artery dilates in response to the physical stress of increased flow.

We recently completed a study that examined whether acute stress impacted the magnitude of arterial blood flow. FMD was measured during a rest and a stress period in participants over time increments (baseline, 10 min, 45 min, and 90 min). The results of this study showed that FMD was lower during the stress testing session than during the rest testing session, but significant reductions in FMD at 90 min post-stress were not present (Krick 2004). However, in our preliminary analyses, we did not measure the magnitude of the flow stimulus across the 4 time points. This is important because the size of the flow stimulus is a direct determinant of the magnitude of flow-dependent dilation.

Purpose of the present study

It appears that stress may be involved in the development of atherosclerosis (Strike and Steptoe, 2004). However, the mechanism(s) for this effect are not known. Previous studies have examined changes in forearm blood flow during stress. In general, they find that baseline blood flow significantly increases and that this increased blood flow returns to baseline within 10 min (Lindqvist 2004). However, in studies of FMD it is also interesting to know whether acute stress changes the peak hyperemic flow (the rate of blood flow after the release of a blood pressure cuff). Unfortunately, few papers have examined hyperemic flow after stress. This is due, in part, to use of older technologies for measuring forearm blood flow. In a recent study, Ghiadoni et al (2000) examined changes in forearm blood flow and FMD under resting conditions and at 30, 90, and 240 min post stress. Although they did not show the data on hyperemic flow in the manuscript, they noted that hyperemic flow was not changed in the 240 min after stress (Ghiadoni et al., 2000). Therefore, the present study investigated the effects of brief stressor on brachial arterial blood flow to examine one potential mechanism through which stress affects CVD risk.

Methods

Participants

Subjects for this study were carefully screened to ensure that they were healthy and they met the criteria for inclusion. Subjects were recruited through fliers and by contacting eligible subjects involved in previous studies in Dr. West's lab. After an initial telephone interview, potential subjects were scheduled for a screening visit at the General Clinical Research Center at the Pennsylvania State University campus to assess height, weight, blood pressure, and fasting serum lipids. In total, 29 individuals were recruited 14 healthy subjects (2 women) and 15 hypercholesterolemic subjects (1 woman). Inclusion criteria were as follows: LDL-C and total cholesterol between the 50-90th percentile as defined by NHANES III, triglycerides between 100-350 mg/dl, and age 35-65 years. We enrolled all eligible men and eligible post-menopausal women who were not on hormone replacement therapy (HRT). The following criteria were used to exclude potential participants from participation: current use of lipid-lowering medications (statins, niacin, bile acid binding resins) or anti-hypertensive medications (ACE-

inhibitors, beta-blockers, calcium-channel blockers, diuretics), use of oral or injected corticosteroids (steroid medication), inhaled beta-agonists, atherosclerotic disease, history of MI or angina; vascular disease, including peripheral vascular ischemia, intermittent claudication, retinopathy or significant neuropathy; inflammatory disease; tobacco use (cigarettes, cigars, chewing tobacco); allergy to latex or adhesive tape. It was important to ensure that all participants were healthy, expressing no clinically evident cardiovascular disease or diabetes, so that underlying disorders, such as metabolic syndrome, could be excluded as a confound.

After the screening visit, participants engaged in 1 or 2 additional visits. All participants took part in the stressor testing sessions, and a subset also returned for a rest only testing session. However, this report is restricted to the results of that acute stress session only. Participants were asked to fast for 12 hours prior to testing. Upon arriving at the General Clinical Research Center, participants underwent a blood vessel function ultrasound test (described below). During the speech task, participants were presented with a problematic scenario, and then given 2 min to prepare a 3-min speech. Participants believed they were being videotaped as they presented; however, no actual recording occurred. Upon completion of the stressor portion of the session, participants were asked to rest for 10-min, at which time the second FMD and blood flow measurement was collected. A third FMD and blood flow measurement was collected 45-min following completion of the speech task (15-min after the previous FMD measurement). The final FMD and blood flow measurement was collected 90-min following completion of the speech task. Testing sessions lasted approximately 3-hours, and participants remained in a supine position for the duration of the testing sessions. Studies have shown that posture does not inhibit hemodynamic reactivity to stress, and responses to the speech stressor should agree with previous literature, regardless of posture (Sherwood and Turner 1993; Radaelli et al. 1994).

FMD of the brachial artery and brachial artery blood flow were assessed in the right arm four times during each visit once at baseline, and again at 10 min, 45 min, and 90 min post stress. This assessment was completed under controlled conditions by trained and blinded sonographer. The brachial artery above the elbow of the dominant arm was scanned in longitudinal sections. Changes in diameter were assessed by external B mode ultrasound imaging (using an Acuson Aspen equipped with 10 MHz linear array transducer; Acuson, Mountain View, California). This technique allowed for reliable detection of changes in vascular diameter as small as 0.1 mm. Participants wore a standard blood pressure cuff on the opposite arm and measurements were taken once every other minute during each rest and stress period. All blood flow velocity measurements were collected using pulsed Doppler flow with the probe at a 70 degree angle to the vessel.

Baseline measurements of blood flow, blood pressure, and heart rate were obtained after 15 min of supine rest. A pneumatic tourniquet was placed around the forearm distal to the target artery and inflated to a pressure of 200 mmHg for 5 min. Increased flow was induced by sudden cuff deflation. Images were recorded continuously during cuff deflation and they continued for 120 seconds afterwards.

Ultrasound images were stored on high quality sVHS tapes and analyzed by a single experienced observer. The rate of blood flow (ml/min) was calculated using customized software for measuring velocity time integrals of Doppler flow wave forms as follows [flow (ml/min)= velocity time integral x cross-sectional area of the vessel x heart rate].
Statistical Analyses

All variables were analyzed using a mixed models approach using SAS PROC MIXED (SAS v.8, Cary, NC). Models included condition, time, visit number, and condition order as fixed effects, and subject as a random effect. There were no significant effects of condition order. Tukey-Kramer adjusted *P*-values were used to examine the source of significant effects. Text and tables report least squares means \pm SE, and probability values ≤ 0.05 were considered statistically significant.

Results

Effects of subject characteristics on flow

Examination of the cholesterol status of the participants in this study did not show any differences in flow volume or velocity between the groups. Due to this observation we were able to combine the two groups together and analyze their results as a whole.

Effects of acute stress on flow

Results are presented for six variables: base flow (ml/min), peak flow (ml/min), percent change in flow (%), base velocity (M/s), peak velocity (M/s), and percent change in velocity (%). Each of these variables were examined separately across the 4 time intervals (0, 10, 45, and 90 min post stress). We observed significant main effects of time for baseline blood flow volume; baseline flow velocity, percent change in blood flow volume, and the percent change in blood flow velocity (all *P*'s ≤ 0.003). Figure 1 and Table 1 show that basal blood flow volume (the amount of blood flowing through the vessels before the cuff was inflated) showed significant changes over time. Baseline flow (ml/min) at 10 min post stress was unchanged compared to the resting value. However, at 45 min and 90 min post-stress there was a significant decrease in basal blood flow volume. Peak blood flow (ml/min, measured after the cuff was released) was unchanged at 10 min, 45 min, and 90 min vs. the pre-stress baseline. (Table 1, Figure 2). The percent change in blood flow after cuff release was not changed 10 min post-stress vs. the pre-stress baseline. However, this variable was significantly increased at 45 and 90 min post stress (Table 1, Figure 3).

Similar patterns were seen for measurements of blood flow velocity (thrust and speed of blood flow). The baseline velocity of blood flow (M/s) showed no significant changes in blood flow from 0 min to 10 min post-stress. At 45 min and 90 min post-stress, there was a significant decrease in basal blood flow velocity (Table 1, Figure 4). However, the peak velocity of blood flow (M/s) showed no significant change at 10 min, 45 min, and 90 min post-stress (Table 1, Figure 5).

The percent change of velocity showed no change at 10 min or 45 min, but this velocity measurement did increase at 90 min post-stress (Table 1, Figure 6).

Discussion

Our results suggest that a brief stressor will alter blood flow volume and blood flow velocity, and that these effects are only evident 45 – 90 min post-stress. This is in contrast to previous studies showing that baseline blood flow significantly increases during stress and that this increased blood flow returns to baseline within 10 min (Lindqvist, 2004). Our study showed significant reductions in basal blood flow volume and blood flow velocity at 45 min and 90 min post stress. Peak (post-deflation) blood flow and blood velocity remained unchanged. Therefore, the percent increase in blood flow and blood velocity at 90 min was higher in the post-stress period than under resting conditions.

In addition, our study also showed that participants with high or low cholesterol produce the same magnitude of change in blood flow and velocity after being stressed. Ultimately, our work suggests that acute stressors do have an impact brachial artery blood flow volume and blood flow velocity, that that these parameters need to be monitored for more than 10 min post stress to see this result. One important limitation of this analysis is that we did not include data from the stress-free testing session. Therefore, the changes in blood flow and velocity that we observed at 45 and 90 min post-stress may actually result from subjects continuing to fast during the post-stress period. Future studies must include time-matched control sessions to rule out this possibility.

This study has several other limitations. First, we examined the response to a controlled stressor in the laboratory. It is possible that subject exhibit a different pattern of responses to real stressors encountered in their daily lives. In addition, we may have seen a difference between high and low cholesterol participants if we had studied patients with a wider range of cholesterol levels. Women were under-represented in the sample due to concerns about menstrual cycle fluctuations impacting vascular responses. Therefore, it is not clear whether gender differences exist in blood flow responses to stress. Future studies could address by recruiting more women. Finally, our study was only done when the participant was in a supine position and most stressors are encountered when people are standing or seated; a different position could have different results.

Further studies

We suggest that future studies include a control session and that they assess changes in blood flow for longer periods of time in order to understand how stressor exposure affects vascular function. Researchers may want to recruit representative samples of women and men to see if they show different results. Also, future studies should enroll participants with a wider range of cholesterol levels to test whether patients with more advanced metabolic problems show larger post-stress changes in blood flow

Table 1. Effects of acute stress on blood flow during the 90 minute recovery period. Data are presented as mean \pm SE.

	Pre-stress	10 min Post-stress	45 min Post-stress	90 min Post-stress
Base flow (ml/min) ^a	167.6 \pm 11.5	137.4 \pm 11.5	125.1 \pm 11.5*	122.8 \pm 11.5*
Peak flow (ml/min)	1074.3 \pm 60.7	1081.6 \pm 60.7	1097.2 \pm 60.7*	1115.4 \pm 60.7*
% change flow (%) ^a	681.7 \pm 71.5	787.8 \pm 71.5	876.4 \pm 71.5	944.5 \pm 71.5
Base velocity (M/s) ^a	0.90 \pm .02	0.89 \pm .02	0.84 \pm .02*	0.83 \pm .02*
Peak velocity (M/s)	1.71 \pm .05	1.72 \pm .05	1.72 \pm .05	1.74 \pm .05
% change velocity (%) ^a	94.1 \pm 6.4	95.5 \pm 6.4	106.6 \pm 6.4	112.1 \pm 6.4*

^a Significant main effect of time, $p < 0.05$

* Significantly different vs. the pre-stress measurement (Tukey test $P \leq 0.05$).

Figure 2

Effects of acute stress on peak flow

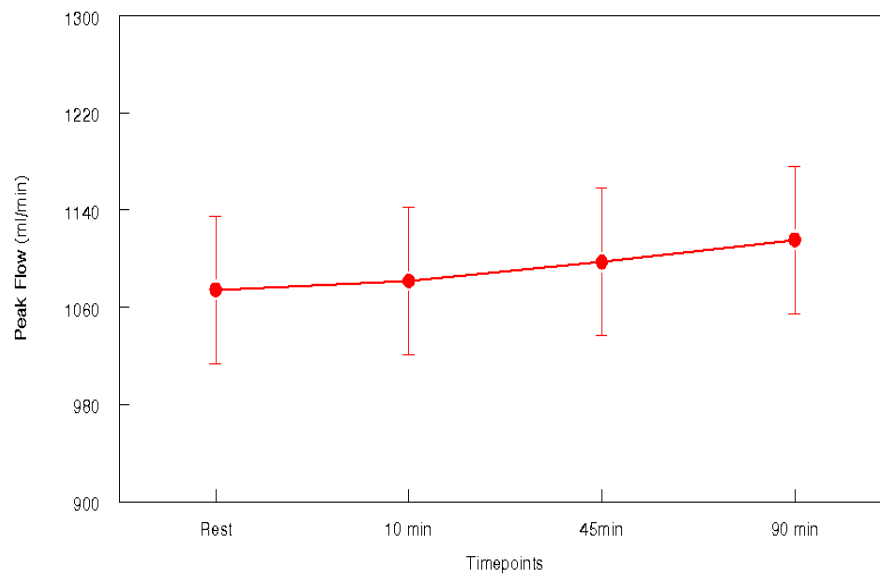


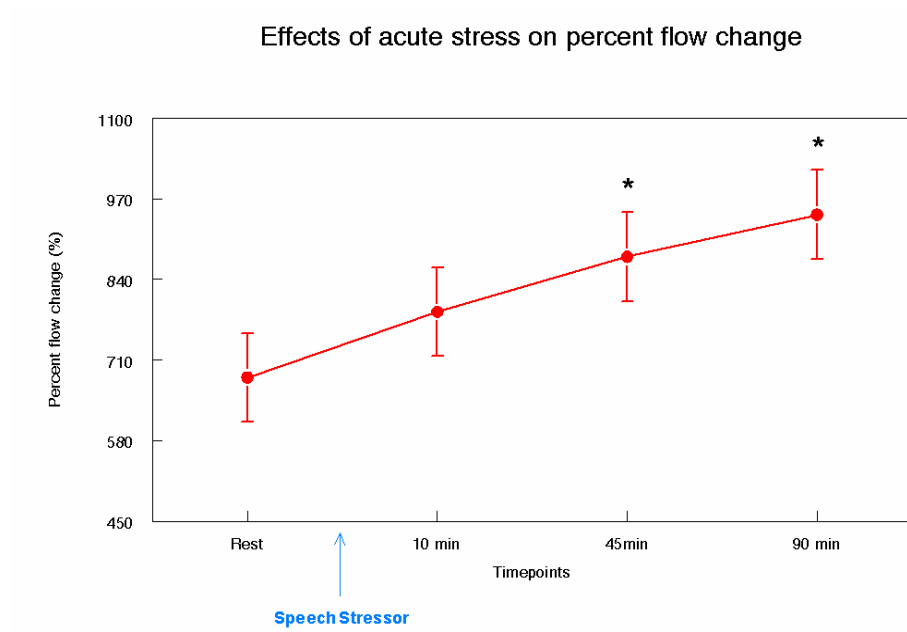
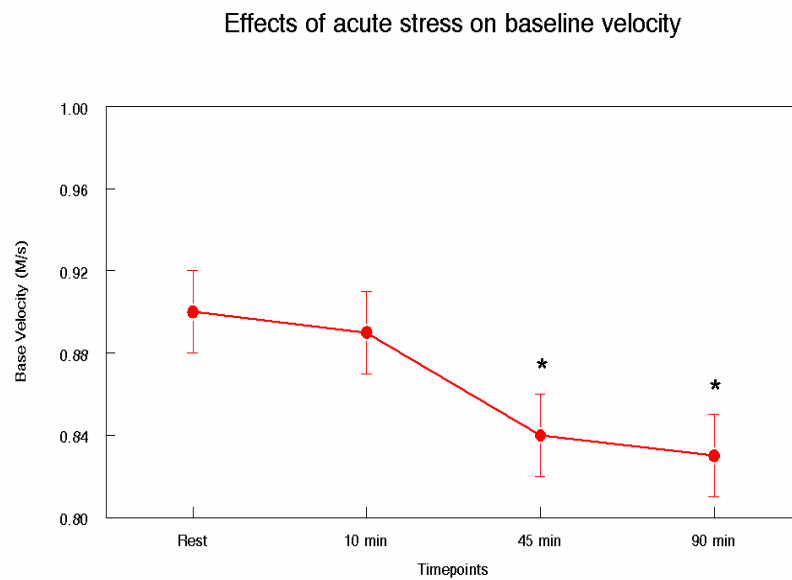
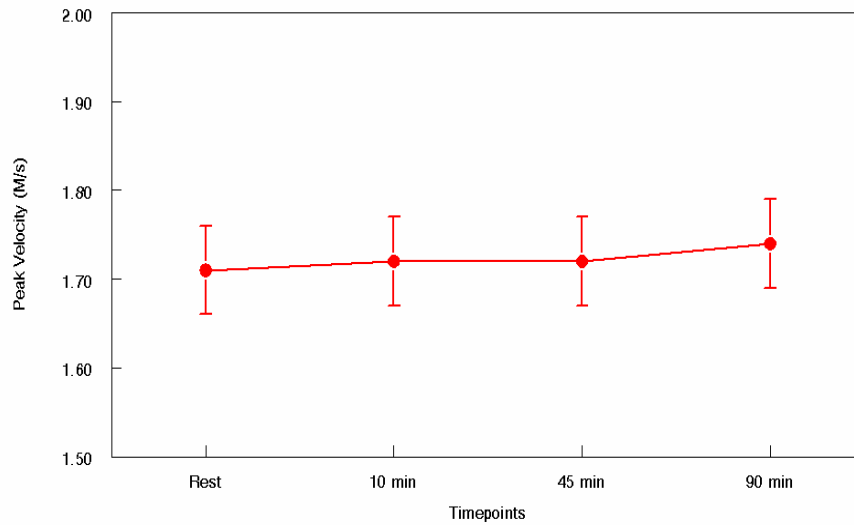
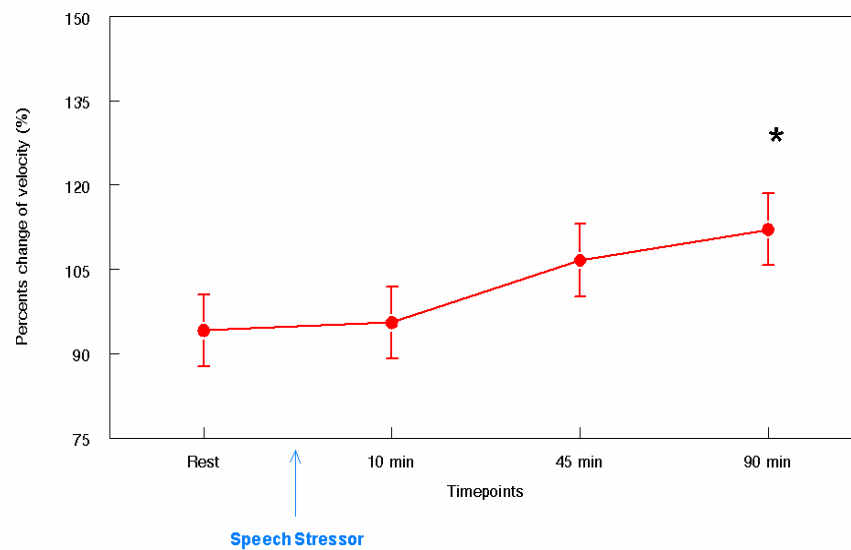
Figure 3Figure 4

Figure 5

Effects of acute stress on peak velocity

Figure 6

Effects of acute stress on percent change of velocity



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Preserving a Woman's Genitalia: An Analysis of Female Circumcision/Female Genital Mutilation in Africa

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Abstract

There continues to be a high prevalence in the rate of female circumcision/female genital mutilation practices in about 28 countries in Africa. Worldwide prevalence estimates that FC/FGM affects over 140 million women, with an additional two million girls and women undergoing the procedure every year. The purpose of this research is to determine the point where one's culture becomes an impediment to one's rights to good health and development. Finding a balance between a society's way of life and the protection of individuals from the violation of their human rights is addressed in preserving women's genitalia.

Testimony

'I was genitally mutilated at the age of ten. I was told by my late grandmother that they were taking me down to the river to perform a certain ceremony, and afterwards I would be given a lot of food to eat. As an innocent child, I was led like a sheep to be slaughtered.

Once I entered the secret bush, I was taken to a very dark room and undressed. I was blindfolded and stripped naked. I was then carried by two strong women to the site for the operation. I was forced to lie flat on my back by four strong women, two holding tight to each leg. Another woman sat on my chest to prevent my upper body from moving. A piece of cloth was forced in my mouth to stop me screaming. I was then shaved.

When the operation began, I put up a big fight. The pain was terrible and unbearable. During this fight, I was badly cut and lost blood. All those who took part in the operation were half-drunk with alcohol. Others were dancing and singing, and worst of all, had stripped naked.

I was genitally mutilated with a blunt penknife.

After the operation, no one was allowed to aid me to walk. The stuff they put on my wound stank and was painful. These were terrible times for me. Each time I wanted to urinate, I was forced to stand upright. The urine would spread over the wound and would cause fresh pain all over again. Sometimes I had to force myself not to urinate for fear of the terrible pain. I was not given any anaesthetic in the operation to reduce my pain, nor any antibiotics to fight against infection. Afterwards, I haemorrhaged and became anaemic. This was attributed to witchcraft. I suffered for a long time from acute vaginal infections."

Hannah Koroma, Sierra Leone (Amnesty International, 2005)

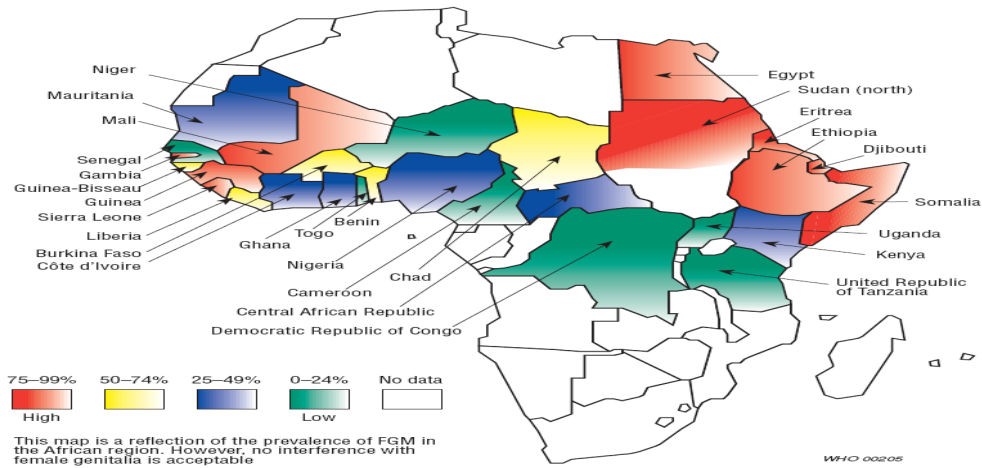
Introduction

For nearly 2,500 years, infants, young girls, and women in some African countries succumb to the traditional act of Female Circumcision/Female Genital Mutilation. Controversies continue to surround the complex cultural and painful act that violates the rights of women and children. The topic of Female Circumcision/ Female Genital Mutilation elicits heated debate between opponents and supporters of the act. Opponents of FGM seek its eradication as they argue that it violates human rights, rights to development, rights to good health, rights of women and children by causing its victims to adhere to lifelong health and psychological complications. Supporters of FGM justify its continuation by emphasizing the importance of maintaining one's cultural tradition as it prepares young children and women for womanhood and marriage and secures one's place in society both socially and economically.

Nonetheless, FC/FGM is a traditional act commonly practiced on young girls and women in some African countries. The World Health Organization (WHO) defines the act, as all procedures, which involve partial or total removal of the external female genitalia or other injury to the female genital organs whether for cultural or any other non- therapeutic reasons. (WHO, 2001) Depending on the degree of severity, the female's clitoris, labia minora or small lips, and the labia majora or large lips are cut or scraped away and in some cases sewn together, leaving only a tiny opening for the passing of urine and menstrual fluid.

Currently, female circumcision is widespread in about 28 countries in Africa. It is important to note that not all groups in Africa practice female circumcision as it is

practiced in twenty-eight out of fifty-three African countries. (Lightfoot-Klein, 1989) In addition, prevalence varies from country to country and from one ethnic group to another. About 90 percent of women in Egypt, Somalia, Mali, Ethiopia, and Sudan undergo this practice. Ethnic groups like the Kikuyu in Kenya, practice excision and the Luo do not; in Nigeria, the Yoruba, Ibo, and Hausa girls and women experience the circumcision of their genitals, but not the Nupes or the Fulanis. (Dorkeeno, 1994)



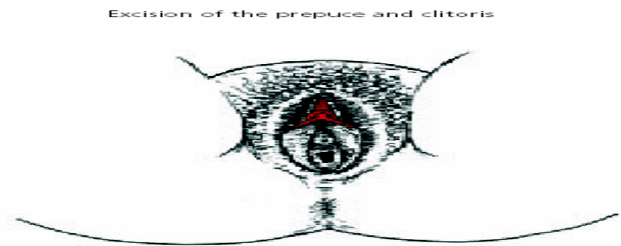
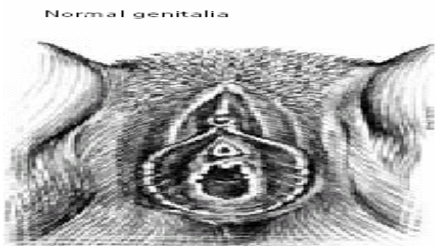
Over 140 million African women have been circumcised, with an additional two million girls and women subjected to the procedure every year. The vast majority of female genital mutilation is excision of the clitoris and the labia minora, accounting for up to 85% of all cases. The extreme form is infibulation, which constitutes about 15% of all procedures. (Amnesty International, 2005) The incidence of infibulation is much higher in Djibouti, eastern Chad, Somalia, central and northern Sudan, southern Egypt, and parts of Ethiopia and Eritrea. (Greubbaum, 2001) These women are at risk for immediate short and long term health and psychological complications resulting from experiencing this traditional practice.

Historians have traced the origins of female circumcision back to as early as the fifth century before the Christian era. The oldest historical source to this act is to be found in the writings of Greek historian, Herodotus, who reported that the Phoenicians, Hittites, Ethiopians as well as Egyptians practiced the custom of Female Circumcision. This traditional practice evolved as a means for protection against rape for young girls taking animals out to the pasture or an early attempt at population control. (Lockhat, 2004) According to Hanny Lightfoot-Klein (1989), various authors have shown that early Roman and Arabs practiced female circumcision. "In some groups it appears to have been a mark of distinction, in others a mark of enslavement and subjugation." (Klein, 1989) Furthermore, the practice of cutting a woman's genitalia resulted from primitive man's desire to gain mastery over the mystery of female sexual function. By excision of the clitoris, sexual freedom in women is curbed and women are changed from common to private property, the property of their husbands alone. (Klein, 1989)

Descriptions of the various forms

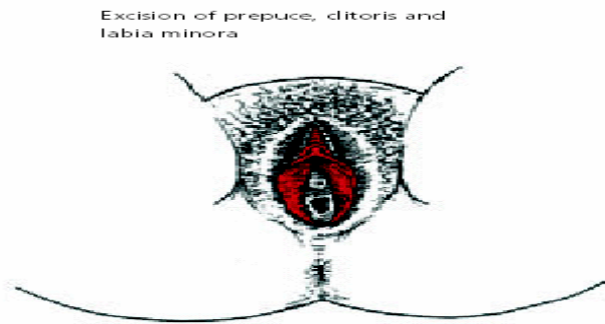
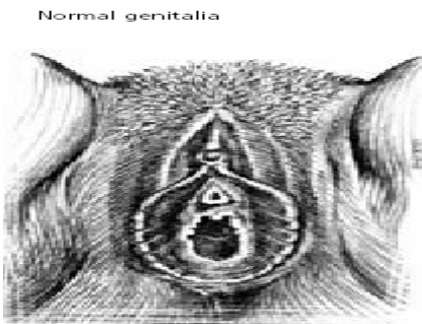
The WHO categorizes Female Circumcision/Female Genital Mutilation into four basic forms depending on the degrees of severity. (WHO, 2001)

Type I: Circumcision or Sunna ranges from excision of the prepuce (the fold of skin above the clitoris) with the tip of the clitoris to complete excision of the prepuce and clitoris



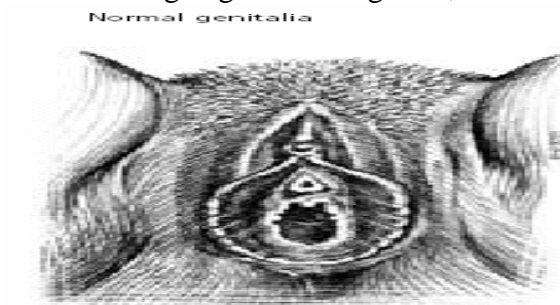
(WHO, 2001)

Type II: Clitoridectomy or Excision of the prepuce, clitoris and labia minora



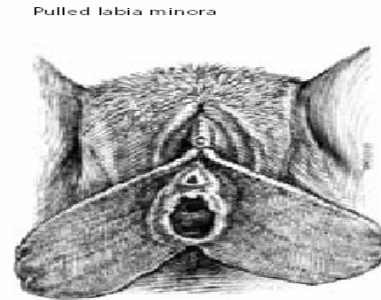
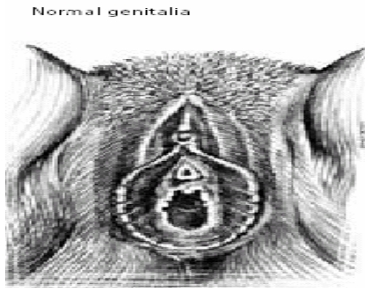
(WHO, 2001)

Type III: Infibulation or Pharonic Circumcision is the most severe form of the practice and it involves the removal of virtually all of the external female genitalia. The entire female clitoris, labia minora, and most of the labia majora are cut or scraped away and the remaining edges sewn together, with a tiny opening left for urine and menstrual fluid



(WHO, 2001)

Type IV: Unclassified: includes pricking, piercing or incision of clitoris and/or labia; cauterization by burning of clitoris and surrounding tissues; scraping (angurya cuts) of the vaginal orifice or cutting (gishiri cuts) of the vagina; introduction of corrosive substances into the vagina to cause bleeding or herbs into the vagina with the aim of tightening or narrowing the vagina; any other procedure which falls under the definition of FGM given above



(WHO, 2001)

The Process and Conditions of the Practice

The age at which females are circumcised varies anywhere from eight days after birth to 14 or 15 years old, depending on the country and particular culture. In countries with female circumcision data, the median age of the females ranges from the eighth day after birth in Ethiopia, to less than two months in Eritrea, between five to eight years in Sudan, 10 years in Egypt, and as late as 14 or 15 in Kenya. In Tanzania, young women are circumcised on their wedding nights, and in Mali, the operation may be performed on married women after they have had their first child.

A traditional birth attendant or elderly women generally perform the operation. In urban areas of some countries, medical professionals usually perform female circumcision in hospitals. The procedure may take 15- 20 minutes depending on the skill of the operator, the type of excision, and the resistance put forth by the girl.(WHO, 2001) The instruments used to perform female circumcision range from household knives, old razor blades, tin lids, sharp stones, broken glasses, and other cutting instruments. Scalpels are used in hospital settings. (Slack, 1988) When infibulation takes place, thorns, cat or lamb intestines may be used to stitch the two sides of the labia majora together. A girl's legs may be bound together for up to two months to facilitate healing. (Amnesty International, 2005) With the exception of hospitals, this traditional act is often performed without any anesthetics, and the instruments used are rarely sterilized.

Reasons and Justification for the Practice

Custom and tradition are the most cited reasons for the practice of FC/FGM. (Amnesty International, 2005) According to Toubia (1994), this ritualistic practice reflects the ideology and cultural values of each community that practices it. Davies (1976) stated that female circumcision "an expression of male power" is a demonic desire to control female sexuality, and endless tyranny of the dominating male behind the defense of culture.

Other reasons and justification for the enduring prevalence of this practice include: socio-cultural reasons, hygienic-aesthetic reasons, religious reasons and psychosexual reasons. In addressing socio-cultural reasons, FC/FGM serves as a rite of passage, a marker of movement from childhood immaturity to adulthood maturity. Baasher (1982) reported that in FC/FGM practicing communities no one will marry an uncircumcised female. The late Jomo Kenyatta, Kenya's 1st president and a member of the Kikuyu wrote that no proper Kikuyu would dream of marrying a girl who had not been circumcised. (Kenyatta, 1953) In countries such as Sudan, Mali, and Kenya, an

elaborate ceremony filled with special songs, dances, and chants is performed to teach young circumcised girls their duties as wives and mothers. The whole event is one of excitement, anticipation, and festivity. The circumcised girls are showered with gifts, money, and new clothes adorned with henna and gold jewelry, and given almost a bride-like status. (Baasher, 1979) Because societies that practice this act are patriarchal and largely patrilineal, a woman's access to land and security is through marriage and only circumcised women are considered suitable for marriage. (WHO, 2001)

For hygienic and aesthetic reasons, societies that practice this act argue that a woman's external genitalia are ugly and dirty. According to Dorkeeno, in Egypt, for instance, the unexcised girl is called nigma (unclean) and bodily hairs are removed in an effort to attain a smooth and therefore, clean body. (Dorkenoo, 1994) Young girls and women in African countries that practice FC/FGM are believed to be naturally polluted and they can only reach a state of cleanliness suitable for marriage and childbirth through excision. (Hosken, 1994)

Also, in addressing religious reasons, FC/FGM is practiced by Muslims, Christians, and non believers in a range of communities. Despite the fact that FC/FGM is not known in many Muslim countries such as Saudi Arabia, Jordan, Iran, etc. it has, however, frequently been carried out by some Muslim communities in the genuine belief that it is mandated by the Koran and demanded by Islamic faith. According to Adeyemo (2003), the ideology that FC/FGM is associated with the religion of Islam is erroneous because as a cultural practice, its prevalence is widespread in countries that are predominantly Christian in their religious beliefs.

Another reason for the practice of female genital mutilation is that circumcision of the genitals ensures virginity, preserves the chastity of a woman and thus maintains family honor. (Assad, 1979) These psychosexual reasons are responsible for the enduring prevalence of FC/FGM in practicing communities, as an unexcised girl is believed to possess an overactive and uncontrollable sex drive, with the likelihood of losing her virginity prematurely, thus bringing disgrace to her family while damaging her chances for marriage. (WHO, 2001) Because the clitoris is believed to drive women into making uncontrollable, insatiable demands for sex, circumcision of this organ minimizes her promiscuity, preserves her chastity, and maintains her family's honor while reducing her likelihood for extramarital affairs. (Lockhat, 2004)

Human Rights versus Cultural Rights

In analyzing Female Circumcision/ Female Genital Mutilation, two major opposing views continue to prevail. While the Human Rights Perspective argues that this practice violates a basic human right of intact genitalia, the Cultural Self Determination Perspective insists that female circumcision represents a cultural rite of passage. Using the Universal Declaration of Human Rights adopted by the UN General Assembly in December 1948, Lockhat (2004) attests that all human beings are born free and equal in dignity and rights. The number of articles relevant to the prevalence of Female Circumcision includes:

Article 3: Everyone has the right to life, liberty and the security of person.

Article 5: No one shall be subjected to torture or to cruel inhuman or degrading treatment or punishment

Article 15: Everyone has the right to a standard of living adequate for health and wellbeing of himself.

Based on these articles, opponents of Female Circumcision/ Female Genital Mutilation argue that the practice violates basic human rights, the rights to health, the rights of women and the rights of children.

In contrast, supporters of this practice argue that female circumcision is a tradition that prepares females for womanhood and marriage. Lockhat (2004) suggests that advocates of the practice argue that it is neither inhumane nor degrading and it is not done to exploit or damage the health and well being of the females. Matais (1996) stated that as a traditional practice, FC/FGM is often thought to purify and protect the next generation from dangerous outside influences. As part of intensive group socialization, FC/FGM establishes age set relationships, generational respect and authority patterns. Other functions of the traditional practice include, control of female sexuality and marital chastity. FC/FGM ensures marriage in a society in which men have been taught that only circumcised women make good wives. Nonetheless, it is said that the three most difficult and yet joyous times in a women's life are at her circumcision, marriage, and on the birth of her first child as they mark her transition from one stage of life to another. (African Update, 1996)

The heart of these two controversial views lies in finding a balance between a society's way of life and the protection of its individuals from the violation of their human rights.

Purpose of Research

The goal of this research is to explore one's cultural rites of passage verses the violation of human rights. Utilizing past research, the point where one's culture becomes a violation of their human rights is analyzed. Arguments in support and against Female Circumcision are also explored to understand the occurrence of the practice on infants, young girls, and women. Opponents of the practice argue that FC continues to perpetuate because it is practiced in patriarchal societies where men subjugate women. In these societies women have low status educationally and economically. Despite the detrimental health and psychological implications of the act, Female Circumcision continues to prevail because it is used to control the sexuality and marriage ability of females. Calder (1993) attests that women's sexuality is controlled because excision of the clitoris decreases sexual desire and pleasure, and guarantees virginity until marriage.

Proponents of the practice argue that Female Circumcision is a cultural practice used as a rite of passage to prepare young girls for womanhood and marriage. It also guarantees the virginity of young females until marriage. Althaus (1997) confirms that a girl's virginity is considered essential to her family's ability to arrange her marriage and receive a bride price. Female Circumcision ensures the economic future of a girl child by guaranteeing a bride price.

Nonetheless, this research explores theories of social change, health perspectives, psychological perspectives, anthropological perspective, arguments against the practice from human rights perspective, and arguments in support of the practice using a cultural rights approach. Acceptable interventions and prospects for the future are also analyzed in this research to find a balance between a society's way of life and the protection of individuals from the violation of their human rights.

Methodology

Using an interdisciplinary approach, this research analyzes female circumcision/female genital mutilation qualitatively by conducting a literature review. Books, peer reviewed journals, newspaper articles, and non-governmental sources, are explored to understand the cultural rites of passage and violation of one's human rights.

The research efforts of many different individuals such as Fran Hosken, Hanny Lightfoot- Klein, Haseena Lockhat, Nahid Toubia, Efua Dorkeeno, Esther Ogunmodede, Ellen Gruenbaum, Daniel Gordon, Alison Slack; in conjunction with joint statements from World Health Organization, United Nations Children's Fund, United Nations Population Fund; organizations such as Amnesty International, Minority Rights Group and articles from *Human Rights Quarterly*, *African Update*, *Medical Anthropology Quarterly*, *International Family Planning Perspectives*, *Journal of Health and Social Behavior*, *Journal of Marriage and Family*, *Health Care for Women International* were used to explore cultural rights of passage and the violation of human rights.

Understanding Female Circumcision/ Female Genital Mutilation requires much more than merely knowing the facts, therefore an interdisciplinary approach using descriptions, narratives, and examples from many different sources is used in this research. Theories of social change, health perspectives, psychological perspective, anthropological perspective, arguments against the practice from a human rights perspective, and arguments in support of the practice from a cultural rights perspective are analyzed. The information sought examines the point where one's culture becomes a violation of human right.

Theories of Social Change

Three theories of social change are used to examine, the practice, prevalence, and persistence of mutilating a woman's genitalia. According to Yount (2002), modernization theory, convention theory and feminist theory offer three different explanations for persistence in the practice of FC/FGM.

Modernization theory argues that female genital mutilation perpetuates in partilineal societies whereby male family members inherit land and women acquire access to resources through marriage. Nonetheless, modernization theory predicts that increased urbanization, education, wage labor, communication, and economic development will reduce the value of landed inheritance, alter attitudes about marriage and women's position and thereby weaken the practice of female genital mutilation. (Kennedy, 1970; Hayes, 1975)

Evidence about the role of modernization is varied. Although urban residence is negatively associated with female genital mutilation and its perceived necessity in Egypt and southwestern Nigeria (Yount, 2002), Hicks (1993) has attributed the absence of change in some urban areas to persistent socio-economic ties with rural settings. The absence of these changes can lead to close ties with families in rural areas for sources of support, such as child care, food supplies from cash crops, or economic resources. These ties are then responsible for the prevalence of this practice in both urban and rural settings. In addition, Yount (2002) attests that certain aspects of modernization such as medicalization of practice and the use of anesthetics in hospital settings have also been associated with the adoption of less severe forms of circumcision.

Convention theory argues that conditions of resource inequality are responsible for the origination of female genital mutilation. According to Yount (2002), 'in societies whereby high ranking men attract multiple potential wives, families, become motivated by competition to enforce codes of modesty upon their daughters.' (Yount, 2002) Mackie (1996, 2000) stated that the main force perpetuating the custom is the link between female genital mutilation and marriage ability. The maintenance of female genital is a conventional sign of marriage ability that is universal. Women in FC/FGM communities, who support the practice, are caught in a self reinforcing belief trap or a belief that cannot be revised because the perceived costs of testing it single-handedly (e.g, loss of family honor, unmarriageable daughter) are excessive. (Mackie 1996, 2000) Nonetheless, Mackie (2000) suggests that marriage ability is a vector of transmission and the main engine for the continuation of FC/FGM in practicing communities.

Ahmadu (1995) states that significance of female circumcision extends beyond marriage ability in the cases of Kono women of Sierra Leone. Reproduction and sexuality are the main reasons for the prevalence of this practice in Kono community as the presence of the clitoris is seen to inhibit female fertility and sexuality. Kono women adamantly believe that if left untouched, the clitoris will continue to grow and become unsightly, like the penis leading to incessant masturbation and sexual insatiability. According to the Kono ideology, masturbation is seen as a deterrent to female fertility. Once a young girl discovers that she can sexually stimulate herself or be manually stimulated by others, she does not desire or seek vaginal penetration by a man's penis. Nonetheless, excision of the clitoris for the Konos would inhibit uncontrolled masturbation in girls and sexual insatiability for women. (Ahmadu, 1995)

Nonetheless, convention theory predicts that the eradication of FC/FGM would occur when the common practice elsewhere of not performing female circumcision is introduced, information about the harmful effects of female genital mutilation is reinforced, advantages of intact genitalia are perpetuated, and ‘pledge associations’ whose members promise not to circumcise their daughters are enacted. (Yount 2002)

Feminist theory offers a third explanation for the persistence of female genital mutilation. According to Yount, female genital mutilation persists because it enables women to define their collective social identity. Yount (2002) attests that the practice allows women to acquire some protection and economic security through marriage. Western feminism offers another explanation for the persistence of the practice. Leonard (2000) states that western feminism argues that female genital mutilation persists because it represents a form of patriarchal control over women’s bodies and sexuality. Women then adhere to a social and moral order that expects them to be silent, subservient and pure. (Leonard, 2000)

Despite the differences in these two explanations, both predict that a change in the prevalence of the practice would occur with economic, educational, and social changes in the opportunities open to women. According to Yount (2002) national and community based studies in Egypt show that better educated women less often approve of FC/FGM and less often decide to circumcise their daughters to the practice.

Health Perspectives

In addressing health perspectives, the occurrence of female genital mutilation has immediate short and long term complications that are grave. Sanderson (1981) suggests that some girls die following mutilation, while for others, the physical effects of excision and infibulation range from little discomfort, to problems, which persist throughout life. Dorkenoo (1994) stated that the health risks and complications of female genital mutilation depend upon the gravity of the mutilation, hygienic conditions, the skill and eyesight of the operator, and the struggles of the child. In Nigeria, for example, Esther Ogunmodede stated that most children become so uncontrollable with bewilderment and panic that accidents occur resulting in the serious mutilation of the girl’s genitals. (Sanderson, 1981)

Short-term complications of all types of FC/FGM include severe pain, bleeding from rupture of the blood vessels of the clitoris, shock, and possibly death. About 15 percent of all circumcised females die of bleeding or infections, other reports estimate that out of 1,000 females who undergo female genital mutilation 70 women die as a result. (Dorkenoo 1994) Slack (1988) suggests that death occurs as a result of excessive bleeding, infections, shock, and other complications. Infections caused by using unsterile cutting instruments are also a very common immediate complication associated with FC/FGM. Rahman (2000) suggests that such infection may also occur within a few days of the procedure if the genital area becomes contaminated with urine and feces.

Septicemia is also a result of infections if bacterium reaches the bloodstream. Fatal cases of tetanus have also been recorded following the mutilation of genitals. A possible additional problem resulting from all types of female genital mutilation is that lasting damage to the genital area can increase the risk of HIV transmission during intercourse, which can lead to the Acquired Immunodeficiency Syndrome (AIDS). (Amnesty International, 2004)

According to Toubia (1993), long-term complications are said to be associated more often with infibulation than with excision or clitoridectomy. According to an article by Amnesty International (2005), infibulation can have even more serious and severe long-term effects: chronic urinary tract infections, stones in the bladder and urethra, kidney damage, reproductive tract infections resulting from obstructed menstrual flow, pelvic infections, infertility, excessive scar tissue, keloids (raised, irregularly shaped, progressively enlarging scars) and dermoid cysts. Althaus (1997) suggests that chronic urinary infection is a result of the inability to pass urine because of pain, swelling, and inflammation following the operation.

Other complications include fistulae (holes or tunnels) between the bladder and the vagina or between the rectum and vagina. (Rahman, 2000) Infertility or sterility can also result if pelvic infection causes irreparable damage to the reproductive organs. According to Mustafa (1966), 20-25% of cases of sterility in northern Sudan are a result of infibulation practices.

Infibulated girls also undergo painful menstruation, as blood cannot escape freely. They may also accumulate blood clots around their vaginal opening. Dr Ollivier (1980) a military doctor in Djibouti described the case of a 16-year-old infibulated girl brought to the hospital with unbearable abdominal pains. She had not menstruated for several months, and had not had intercourse, but her abdomen was swollen and sensitive, with signs of the uterus in labor. He performed a de-infibulation surgery by opening up the scarred vaginal wall and released 3.4 liters of blackish foul-smelling blood. (Dorkenoo 1994)

Common sexual problems range from diminished desire for sex to pain during sexual intercourse, to avoidance of it completely. According to Sanderson (1981), in Sudan, for example, lack of sexual gratification is common and this is due partly to the absence of the clitoris, which contains nerves of vital importance to sexual enjoyment. With infibulation, normal sexual intercourse cannot take place, because the female's vaginal orifice is too small. Sanderson (1981) attests that it can take months before penetration is possible. Sometimes a husband slits the infibulation scar, or summons a midwife to perform the operation in unhygienic conditions. The de-infibulation of the female then results in more bleeding and pain, in addition to severe lacerations and bruising during sexual intercourse. (Sanderson, 1981) These open wounds may increase a woman's susceptibility to AIDS during intercourse. (Slack, 1988) Where vaginal penetration is impossible, anal intercourse takes place and this can lead to tissue damage and distortion to the anal passage.

Complications during childbirth are also unavoidable. According to Lockhat (2004), tight, unopened circumcision scars and heightened sensitivity to the vaginal and vulva areas can make internal examinations and labor very painful. Also the 'scarred and hardened tissue often blocks the birth passage and results in tearing of the vaginal area, excessive bleeding, or a ruptured uterus.' (Slack 1988) Esther Ogunmodede (1981) attests that labor is unnecessarily prolonged for days. Prolonged labor can lead to tearing of the scar tissue in infibulated females, causing excruciating pain as well as bleeding, injury to adjacent structures and fetal distress with sudden brain damage or even mortality. (Lockhat 2004)

Psychological Perspectives

In addressing psychological perspectives, Baasher (1979) suggests that a small number of clinical cases of psychological illness related to genital mutilation have been reported. However, the few studies conducted on FC/FGM psychological effects report anxiety before the operation, terror and fear at the moment of mutilation, and unbearable pain during the procedure. According to Rahman (2000), girls have also reported disturbances in eating, sleep, mood, and cognition shortly after experiencing the procedure. Many girls and women experience fear, submission, or inhibition and suppressed feelings of anger, bitterness or betrayal. Studies from Somalia and Sudan indicate resulting negative effects on self-esteem and self-identity. Edna Isma'il a lecturer of female genital mutilation described how mental complications begin early in life for Somali girls when they hear tales of horror from already infibulated girls who taunt them before they are mutilated. (Sanderson, 1981)

On the other hand, Dorkeeno's research describes references to special clothes and good food associated with the event, to the pride felt in being like everyone else. (Dorkenoo 1994) Amnesty International (2005) accounts that festivities, presents, and special attention at the time of mutilation may mitigate some of the trauma experienced. But the most important psychological effect on a woman who has survived is the feeling that she is acceptable to her society, having upheld the traditions of her culture, preserved her chastity, and made herself eligible for marriage, often the only role available to her. To be different then produces anxiety and mental conflict, as an un-excised, non-infibulated girl is despised and made the target of ridicule without the ability to marry anyone in the community.

Anthropological Perspective

Ellen Gruenbaum and Daniel Gordon offer two anthropological perspectives on the practice of FC/FGM. Gruenbaum explores patriarchy, ritual, and marriage as conditions for the practice and Gordon examines these procedures in a cultural context. These researchers provide an understanding for the enduring practice and prevalence of FC/FGM.

Gruenbaum (2001) attests that there is a correlation between FC/FGM and patriarchy, although it does not offer a sufficient causal explanation for the enduring prevalence of the act. Necessary conditions for the perpetuation of this practice are the social and economic subordination women and children adhere to in patriarchal societies.

According to Gruenbaum (2001), because subordination of women and girls is so common, there is bound to be a strong correlation between patriarchy and female circumcision.

In addressing, the common question, “Why do they do it?” Gruenbaum (2001) suggests that the questions are about manifest functions, what people believe to be their reasons, and what they hope female circumcision will accomplish for them and their daughters. The rituals associated with the practice may play a role in establishing/enhancing gender identity or marking status transitions, but they do not provide a clear understanding on why children undergo a painful and harmful practice that seems to offer nothing positive. In understanding the practice of circumcision, Greunbaum (2001) suggests that maintaining one’s virginity as well as honor, respectability, and morality are vital to women’s marriage ability. Circumcision of the genitals becomes vitally important as it preserves, protects, and maintains a girl’s virginity, honor, respectability, and morality. Because marriage provides a woman with economic and social security, circumcision of genitals prevails despite health complications.

Daniel Gordon’s research on female genital mutilation explores the practice from a cultural context. Gordon (1991) suggests that mutilation of genitals is a rite of passage that serves as a marker of the movement from child to adult, in which the similarity between male and female is removed, permitting a ritual differentiation of the sexes. According to Gordon, the fundamental reason for this practice is that it serves as ‘something of a social puberty, powerfully signifying the young girl’s future passage into sexuality.’ (Gordon, 1991) Adhering to female genital mutilation changes a circumcised girl’s status from being a young girl to a woman, who is now subjected to strict codes of modesty, chastity, morality, respectability, and honor. The rewards for loyalty to the strict codes are honor to the family and improved marriage prospects for the young mutilated girls. (Gordon, 1991)

Human Rights Perspective

Raham (2000) authoritatively defines human rights to include moral and political claims that every human being has upon his/her government or society as a matter of a right and not by virtue of kindness or charity. To view FC/FGM as a violation of the human rights of women and children is to view this practice as an infringement by governments and societies upon their moral and political claims. (Rahman, 2000)

Opponents of female genital mutilation argue that the practice violates the human rights of women and children as well as their rights to development and health. Using a human rights framework, several studies have examined various conventions that place FC/FGM as a violation of women right’s, children’s rights and their rights to health:

In addressing violations of women’s rights, opponents argue that female genital mutilation denies females their sexual integrity. Article 1 of the Women’s Convention (1979) focuses on the rights of women: *the term discrimination against women shall mean any distinction, exclusion or restriction made on the basis of sex which has the effect or purpose of impairing or nullifying the recognition, enjoyment or exercise by*

women, irrespective of their marital status, on a basis of equality of men and women of human rights and fundamental freedom in the political, economic, social, cultural or civil field.

To view FC/FGM as a basis for discrimination against women, this practice meets two principal criteria of the terms of Article 1: first female circumcision is a distinction, exclusion and restriction based on sex and secondly it has the effect of hindering a woman's ability to enjoy her human rights equally to men. Rahman (2000) attests that FC/FGM falls within the criteria of Article 1, because the practice, reserved for women and girls, has the effect of hindering their enjoyment of fundamental rights. FC/FGM as a matter of gender discrimination, is supported by Article 5 of the UN Convention on the Elimination of all forms of Discrimination against Women (CEDAW 1979) which upholds the need to:

modify the social and cultural patterns of conduct of men and women, with a view to achieving the elimination of prejudices and customs and all other practices which are based on the idea of the inferiority or the superiority of either of the sexes or on stereotyped roles for men and women.

Nevertheless, citing evidence from these conventions, opponents of FC/FGM argue that the practice violates women's rights by causing its victims to adhere to gender discrimination. Since a woman's ability to enjoy her natural physical female characteristics or obtain sexual fulfillments is hindered on the basis of gender discrimination, the practice can be characterized as a violation of human rights.

In addressing the violation of children's rights, Lockhat (2004) suggests that any form of FC/FGM practiced on children constitutes a violation of the rights of that child. According to Rahman (2000), because children cannot adequately protect themselves or make informed decisions about matters that may affect them for the rest of their lives, human rights law grants them special protection.

The Declaration of the Rights of the Child, adopted in 1959 by the General Assembly of the United Nations states that:

Article 2: The child shall enjoy specific protection and shall be given opportunities and facilities by law and other means, to enable him to develop physically, mentally, morally, spiritually and socially in a healthy and normal manner and in conditions of freedom and dignity.

Article 12 upholds: The rights of the child to the enjoyment of the highest attainable standards of health and to medical and rehabilitation facilities.

In addition, The Children Rights Convention (1989) upholds that governments should protect the best interests of the child. Articles in the convention that address the welfare of the child include:

Article 2: States parties shall respect and ensure the rights set forth in the present

convention to each child within their jurisdiction without discrimination of any kind, irrespective of the child's parents, legal guardian's race, color, sex or language.

Article 3: In all actions concerning children, whether undertaken by public or private social welfare institutions, courts of law, administrative authorities' legislative bodies, the best interest of the child shall be a primary consideration.

Article 6(2): States parties shall ensure to the maximum extent possible the survival and development of the child.

In citing these articles, opponents of the practice argue that FC/FGM violates the rights of infants and young children. The UN Declaration of the Rights of the Child and The Child Rights Convention state that every child should have the ability to develop physically, in a healthy and normal manner. However, circumcised infants and young girls are afflicted with health and psychological complications. Their rights to good health and development are denied to them without their consent in the name of a tradition.

Furthermore, in addressing the violation of the rights to good health, Slack (1988) suggests that FC/FGM can be seen as a violation of the right to health because women and children are circumcised in unsanitary conditions without the use of any anesthesia. These females endure debilitating pain, shock, and even death as their genitals are mutilated. Their rights to good health are violated because they endure immediate and long-term severe/chronic health complications that are detrimental to their well-being. In addressing the rights to good health, The Universal Declaration of Human Rights Article 25 states that: *Everyone has the right to a standard of living adequate for the health and well being of himself and of his family.*

In addition, The International Covenant on Economic, Social and Cultural Rights, Article 12 upholds: *the right of everyone to the enjoyment of the highest attainable standard of physical and mental health.*

Because the health and psychological complications associated with FC/FGM can have an overwhelming effect upon a child and woman's physical and emotional health, the procedure can be viewed as a violation of their right to good health. The mutilation of a woman's genital deprives her from enjoying 'the highest attainable standard of physical and mental health,' guaranteed by the International Covenant on Economic, Social and Cultural Rights.

Cultural Rights Perspective

For over 2000 years, many women and children in 28 practicing African countries have experienced the circumcision of their genitals. It is important to understand that these African societies exist as networks of mutually interrelated and dependent groups, emphasizing community rather than self and the individual. According to an article in African Update, the rights of individuals in FC/FGM practicing countries

are not isolated questions and are not normally asserted against group interests because traditionally, the group protects the individual. (African Update, 1996)

Nonetheless, in practicing communities, FC/FGM is seen as a collective experience because it provides a clear and essential function as a way of life. Daniel Gordon (1991) suggests that the operation is a rite of passage, because it serves as a marker of the movement from childhood to adulthood, signifying a young girl's future passage into sexuality. Ifeyinwa Iweriebor points out that the practitioners do not perform genital surgery on their girls to harm them, but rather they engage in the activity for "the noblest of reasons." (African Update, 1996) Iweriebor suggests that in some cultures, FC/FGM serves as a component of a rite of passage to socially acceptable adulthood. For others it represents a nuptial necessity, a mark of courage (particularly when it is carried out on older people), a reproductive aid, and an enhancement of sexuality while increasing fertility. 'Many parents want FC done on their daughters because it protects them from would-be seducers and rapists.' (African Update, 1996)

Nonetheless, the rationale and validation for the procedure as a cultural practice include: sexual control of females, marriage ability, economic security, and tradition. The importance of maintaining one's tradition is a widely held justification for the continuation of the practice. In preserving a woman's genitalia, one needs to understand that FC/FGM is a deeply rooted cultural practice that perpetuates beyond health complications or human rights violation. Proponents of the practice purport that children and women will continue to adhere to female circumcision as a rite of passage because it represents one's way of life. According to Slack (1988), when 280 men and women in Nigeria were given questionnaire surveys about their experiences with the practice, maintaining tradition was the prevailing reason given for the continuation of the act.

In addition, several declarations such as The Universal Declaration of Human Rights suggests, that the right of people to participate in their culture is a human right: *Article 27(1): Everyone has the right freely to participate in the cultural life of the community.* Also, The Declaration of the Principles of International Cultural Cooperation suggests that: *Article 1: Each culture has a dignity and value which must be respected and preserved.* Citing these articles, advocates of this practice argue that FC/FGM practicing communities have a right to adhere to their way of life because the traditional act represents a form of socialization into cultural values, a connection to family, community members and previous generations, maintenance of community customs, and the preservation of one's cultural identity. (Amnesty International, 1997)

Because, the practice of FC/FGM serves as a rite of passage, proponents argue that this transitions young girls from childhood to adulthood while equipping them with skills for marriages and childbirth. According to Rahman (2000), the process of becoming a woman thus contributes to the maintenance of custom and tradition by linking young girls to the lifestyle and roles played by other women. For Kono women, Ahmadu (2000) suggests that the positive aspects of the practice include the initiation ceremony and the celebration of women's preeminent roles in history and society. Among the Kono, a woman is a woman by virtue of the fact that she has been initiated as

the ceremony has the positive value of creating sameness among all women and maintaining equality within age groups. (Ahmadu, 2000)

Acceptable Intervention and Prospects for the Future

In addressing acceptable interventions and prospects for the future of millions of infants, young girls, and women in countries that practice this act, responses and initiatives aimed at slowly changing the practice of FC/FGM are ongoing.

Measures have been taken to put an end to FC/FGM on an international and local level. In Ethiopia, the Ministry of Education implemented educational radio broadcasts to warn its citizens about the detrimental effects of FC/FGM. The National Committee on Traditional Practices in Ethiopia, a committee that includes UN agencies and UNICEF, sponsors the broadcasts. The committee has taken a broad range of activities including: the production and distribution of teaching materials, awareness raising workshops, and awareness raising symposia for media and research. This action, along with a government ban on FGM, is slowly eliminating the practice. (Dorkeeno, 1994)

Also, in Somalia, the SWDO (Somali Women's Democratic Organization) in conjunction with the AIDOS (The Italian Association for Woman and Development) and fully supported by the Somalian government, launched a national campaign to eradicate FC/FGM. While the SWDO was responsible for the content and direction of the campaign, the AIDOS provided technical and methodological support. Information packages were developed to distribute to women, young people, religious leaders and medical professional. These were followed by seminars for women on strategies to bring about change. In addition, the government of Somalia, in collaboration with UNICEF has supported a series of awareness raising seminars calling on women grassroots organizations, religious leaders, health professional and politicians to develop polices on eradication of the practice. (Rahman, 2000)

The UN has also helped fund programs in Sudan where Dr. Amna Abdel Rahman has been working through the Sudan National Committee on Harmful Traditional Practices (SNCTP) to eradicate FGM. Using education materials, SNTCP works with policy makers, men and women organizations, local authority officials, religious, and community leaders, to provide information about the health effects of FC/FGM. In addition, SNTCP undertakes training and information campaigns for local midwives and traditional birth attendants, perpetrators of the practice. (Dorkeeno, 1994)

In Nigeria, the National Association of Nigerian Nurses and Midwives (NANNM), trains health workers to identify the different types of FC/FGM and the harmful effects associated with them. A number of methods such as educational campaigns in markets, televised drama, visits to social and community groups, meeting with traditional and religious leaders to teach people about this harmful traditional practice have been utilized. Also, the Federal Ministry of Health sponsors public awareness and education projects to inform communities about health hazards associated with FC/FGM. (Lockhat. 2004)

Kenya National Committee on Traditional Practices founded by the United Nations Children's Fund (UNICEF) and the United Nations Development Programme (UNDP) work in collaboration with other organizations including WHO, The International Federation of Women Lawyers (FIDA) and Maendeleo Ya Wanawake (MYWO) to eradicate FC/FGM in Kenya. (Lockhat, 2004) Using educational based approaches and radio campaigns, efforts have been focused on informing the community on the dangers of the practice to developing alternative initiation rites of passage that emphasize positive cultural and traditional rituals without incorporating female genital mutilation. (Afrol News, 2005)

In Mali, the Association Malienne pour le Suivi et l'Orientation des Pratiques Traditionnelles (AMSOPT) has carried out projects on the harmful effects of FC/FGM with youth, religious leaders, and excisior throughout Mali. Representatives of the Ghana Association for Women's Welfare (GAWW) speak in communities about the practice and the organize workshops on the female circumcision. Furthermore, numerous actions mounted to preserve a woman's genitalia include; international efforts, legislation, education economic improvement and campaigns.

International Efforts

Dorkeeno suggests that FC/FGM is an international issue because it exists in dozens of countries amongst various ethnic groups. The practice also transcends international boundaries as millions of circumcised girls and women have been forced to flee their countries to become refugees or immigrants in receiving countries.

International organizations such as UN, WHO, Amnesty International, Minority Rights Group, have also stated that female circumcision is a human rights issue because it violates the rights of children and women. Because FC/FGM damages the health of infants, young girls, and women, their right to the highest attainable standard of physical, sexual, and psychological health is violated.

International efforts incorporate numerous international conventions and declarations that address protecting the rights of girls and women, their right to good health and the elimination of female genital mutilation as stated in: The Universal Declaration of Human Rights (1948), The International Covenant on Economic, Social and Cultural Rights (1966), The Convention on the Elimination of All Forms of Discrimination against Women (1979), The Convention on the Rights of the Child (1989), The Declaration on Violence against Women (1993) and The Platform of Action of the Fourth World Conference on Women (1995).

Utilizing the mandates from these conventions, UN Specialized Agencies work with women and governments in FC/FGM practicing countries to create awareness in the subject of mutilating women's genitals. These agencies promote, provide technical assistance and mobilize resources for national and local groups that will initiate community based activities aimed at eradicating the practice. (WHO, 1997)

WHO has adopted clear national policies aimed at eradicating FC/FGM. In addition, general education for the public with emphasis on the dangers of the practice, educational programs for traditional birth attendants and other practitioners have been intensified by WHO to demonstrate the harmful effects of FC/FGM. (Dorkeeno, 1994) In addition a joint statement declared by WHO, UNICEF AND UNFPA is to support global, national and community efforts for the elimination of FGM in order to achieve health and well being for women, girls, their families and communities. (WHO, 1997)

Amnesty International (1997) stresses the relevance of a human rights approach to work against FGM. AI's central goal is to contribute its expertise and experience in human rights campaigning, advocacy and education, as well as its strength as an international and independent mass-membership organization with an increasingly strong presence in Africa, to support and complement the activities of other organizations and individuals who are working towards the eventual eradication of FGM. In addition, organizations like Minority Rights Group, an international research and information unit, support African women campaigning for the eradication of FC/FGM. Foundation for Women's Health Research and Development (FORWARD International), an independent international organization, promotes awareness of FGM, using TV and radio as a medium. FORWARD International acts as an enabler for community organization's grassroots projects, by providing training and educational materials for women working to eradicate the practice in their communities.

Nonetheless, the mission of preserving women's genitalia from a tradition with many harmful health complications would continue to preserve when international organizations adopt strong policies that enforce member countries to develop legislative actions that clearly abolish female genital mutilation. World Health Organizations, United Nations, United Nations Commission on Human Rights, Amnesty International should continue to develop properly planned and well funded programs that focus on finding practical and localized solutions at the grassroots level. These programs should relentlessly educate and create awareness of the health complications associated with the practice. International agencies would also coordinate research, to become a vital resource of factual data and established methods of preserving a woman's genitalia.

Legislation

In addressing legislative actions, Sudan is the first country in Africa to legislate against FGM. The Sudanese government opposes infibulation-type circumcision affirming that it is contrary to the teaching of Islam. In Kenya, the prohibition of FC/FGM is one of the measures contained in the Children's bill passed by parliament in 2001. Female genital mutilation is also banned through Ethiopia's 1994 Constitution, which prohibits harmful traditional practices.

Furthermore, in December 1997, Egypt's highest court of appeals upheld a government banning of the practice, prohibiting all medical and non medical practitioners from performing FC/FGM in either public or private facilities. Since 1996, FGM has been banned in Burkina Faso. President Rawlings of Ghana issued a formal declaration

against FGM and other harmful traditional practices. In 1994, Ghana's parliament amended the criminal code to include FGM as a specific offense. A law passed in 1999, illegalized FC/FGM as a criminal act in Senegal, punishable by a sentence of one to five years in prison. Opponents of the practice in Nigeria, rely on Section 34 (1) (a) of the constitution of the republic of Nigeria which states that; *no person shall be subjected to torture or inhuman or degrading treatment*, as the basis for banning this practice.

Despite these legislative actions in some African countries, other countries like Eritrea, Uganda, Cameroon, Gambia, Benin, do not have laws explicitly against the practice of FC/FGM. In addition legislation is though to have had little effect in rural areas. Nonetheless, Slack (1988) suggests that legislative actions in conjunction with active consistent support and enforcement by each government would derail the prevalence of female circumcision. Because the rights of children and women have ample support in international law, Rahman (2000) stated that governments are bound not only to refrain from violating rights but also to ensure that rights are universally enjoyed in their jurisdictions. Governments have the right to enforce laws to change discrimination against women, abolish practices that are harmful to children and ensure health care.

The duty of the government to modify customs that discriminate against women has been stated in The Women's Convention (1979): *Article 2: State Parties undertake all appropriate measures, including legislation, to modify or abolish existing laws, regulation, customs and practices which constitute discrimination against women.* The duty of the government to abolish practices that are harmful to children have been addressed in The Children's Rights Convention: *Article 24: State parties take all effective and appropriate measures with a view to abolishing traditional practices prejudicial to the health of children.*

In addition it is the duty of the government to ensure health care as stated in the African Charter: *Article 14: State Parties shall take measures to ensure the provision of necessary medical assistance and health care to all children with emphasis on the development of primary health care.*

Citing this article, it becomes imperative for governments to enact laws aimed at changing discriminatory practices, abolishing practices that are harmful to children, ensuring good health and protecting the human rights of infant, young girls and women. The enactment of laws to protect girls and women would guarantee that people understand that FGM is wrong. Laws enacted make it possible for FC/FGM to be classified as human rights violations for women and children and their rights to good health. Rahman (2000) suggests that government actions can create a political and legal environment that deters people from practicing FC/FGM.

Understanding that passing laws will not be enough on its own to protect girls and women from FC/FGM, governments should enact laws to go in conjunction with community education to raise awareness of the health complications and to change attitudes of the practice. Laws against the practice will function effectively with the

improvement a similar system of child protection to inform the against the female circumcision and assist in the enforcement of the legislation

Education

In addressing educational measures, governments should devote resources to supplying educational information to FC/FGM practicing communities about this practice, its harmful effects and human rights in general. The educational resources provided by the government should include information about the potential physical and psychological complications associated with the mutilation of genitals. In preserving a woman's genitals, governments should provide practicing communities with concise educational materials that demonstrate how the practice violates the rights of women and children and their rights to good health.

Also, Rahman (2000) suggests that a form of participatory education, which encompass; literacy training, analytical skills, problem solving, health information and human rights principles should be provided to women over time, in countries that practice circumcision. This approach would seek to empower women and provide them with information about the harmful health complications of the procedure in other to enable them abandon practicing FC/FGM. With enough information to empower themselves, these women would then urge their governments, to make public declaration to denounce the mutilation of one's genitals.

Nonetheless, some countries have adopted education measures as an effort to eliminate FC/FGM. In Nigeria, the African Men United Against Female Circumcision (AMUAFC) educates men and women about the significant health complications associated with FC/FGM. In Egypt, the Ministry of Education uses educational radio series broadcasts to inform people about the harmful effects of FC/FGM. The Babiker Badri organization in Sudan uses teaching methods such as questions and answer booklets and posters to educate Sudanese women about the deleterious effects of FC/FGM. The Ministry of Education in Kenya, which oversees the Federal Institute for Curriculum Development and Research, has made it a requirement to include educational materials discouraging harmful traditional practices, including FC/FGM in primary school curricula. Sinim Mira Nassigue, a Non Governmental Organization (NGO), was established by a group of Guinean men and women. They conduct educational seminars on the deleterious effects of FC/FGM. Also, in Senegal, The NGO Tostan wit the support of the United Nation's Children's Fund began a non formal educational program for women in more than 450 villages. One of its aims was to encourage the empowerment of women. Using games, small group discussions, theatre, songs, dance, story telling, and flip charts, women are taught literacy skills, problem solving skills, women's health and hygiene, management skills, leadership skills, and human rights.

Economic Improvement

In countries in which FC/FGM is a prerequisite for marriage, women and girls whose economic security depends on their ability to be married have little choice but to undergo the mutilation of their genitals. Interventions on the local level by governments, should include polices that enable women to raise their economic status and generate

more income. These policies should ensure that both women and men have the right to work and the right to equal pay for work. Governments should create policies that ensure equal access to education and training for girls, and participation in public office and decision making for women. (Rahman, 2000)

In attempts to eradicate FC/FGM, some countries have adopted economic improvement of women as an action against all forms of FC/FGM. The Kembatta Women Self-Help Center in Ethiopia empowers Kembatta women and their communities and supports their rights to be free from harmful customary practices such as FC/FGM. The center provides vocational training and advancement of women entrepreneurial skills to provide economic improvement for Kembatta women. In Kenya, the Maendeleo Ya Wanawake Association (MYWO, advance the standard of living of life of Kenyan women, by providing measures such as vocational training to enhance the welfare of women and their families. The Tanzania Media Women's Association (TAMWA) has consciously and tirelessly worked to uplift the status of women in society by informing and highlighting the issues and problems which act as barriers to emancipation as full and equal members of the society. This has been done through research work, meetings and seminars, news reports and features, radio and television.

Campaigns

The most important and pleasing development in the eradication of FC/FGM is that African women and men are leading campaigns for its abolition in their communities. In Cameroon, the Ministry of Women Affairs has conducted a campaign against traditional practices that are harmful to women, including FC/FGM. The ministry participates in and supports educational campaigns organized by women's groups working in FC/FGM practicing communities.

In Central African Republic, the Ministry of Social Welfare and NGOs including Women, Nutrition and Development have been involved in awareness building campaigns aimed at eliminating the practice. The Mutawinat Group in Sudan conducts awareness campaigns to eradicate the practice of FC/FGM among Sudanese women. The SWDO (Somali Women's Democratic Organization) and the AIDOS (The Italian Association for Woman and Development) implement campaigns in Somalia to eradicate FC/FGM practices. Also, the Gambian Committee against Traditional Practices (GAMCOTRAP) uses campaigns to inform the public about the harmful effects of traditional practices including FC/FGM. These campaigns provide information on the harmful effects to women, community leaders, youth and children. But campaigns have not been successful in countries like Senegal. The Campaign Pour L'Abolition des Mutilations Sexuelles (CAMS) wanted to use campaigns and organized seminars to galvanize grassroots feminist women's movements to eradicate FC/FGM. This project collapsed due to financial difficulties.

Nonetheless, local governments can foster these campaigns by providing financial and educational resources NGOs. With increased resources, African men and women continue to embark on awareness raising campaigns aimed at providing educational

information, economic improvement and empowerment of women. Campaign programs will specialize in developing health educational materials that inform communities about the complications of FC/FGM.

Conclusion

These acceptable interventions and prospects for the future provide little evidence that the practice of female genital mutilation will decline substantially in the near future. Given the lack of enforcement of most laws against FC/FGM or the resistance put forth by proponents of the practice or the minimal aid from international organizations to eradicate this act, it is unclear whether these measures would eliminate this deeply ingrained cultural practice. Nonetheless, the fundamental question is to assess whether FC/FGM, as a cultural practice violates one's human rights, rights of children, rights of women and rights to good health. Slack (1988) suggests that the controversy boils down to one of tradition versus health- the right to carry on a tradition versus the right to protect infant, young girls and women from debilitating pain, shock, health complications, psychological trauma, permanent bodily damage and even death.

Opponents of FC/FGM define the act as a complex deeply rooted traditional practice that infringes the rights of women and children, proponents suggest that the practice is a fundamental part of a collective cultural experience that relates to the essence of a girl's womanhood, family honor, economic prosperity, and social identity. Because 6000 infants, young girls and women undergo this procedure every day, acceptable intervention should continue to utilize international efforts, legislative laws, economic improvement projects, awareness raising campaigns, and education efforts, to preserve a woman's genitals. The fundamental goal of these measures is to find a balance between a society's way of life and the protection of individuals from the violation of their human rights.

As Toubia points out, 'clear policy declarations by local government and professional bodies are essential to send a strong message of disapproval.' (Toubia, 1993) The greatest achievement of all is to empower African women to lead the campaigns themselves. Governments can provide these women with resources and tools for self-empowerment, economic security, income generation, health services, literacy training and human rights principles.

Because the limitation associated with this research is the inability to conduct the occurrence of FC/FGM in practicing communities, further studies should conduct field research to explore the prevalence of this act within the 28 African countries. Further research should also analyze mediating factors such as mother's circumcision status, rural and urban residency, socioeconomic dependency of women on men, father's literacy level to understand their role in the high prevalence of FC/FGM. Male attitudes towards this traditional practice should also be addressed by future research in attempts to campaign against the genital mutilation of infants, young girls and women. Also, the proposed acceptable interventions and prospects for the future should be accessed and evaluated to examine their impact in preserving a woman's genitalia.

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***Are We Getting the Whole Picture?
The Absence of Crime Data in Newspaper Reporting of School
Violence***

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Abstract

The purpose of this research is to explore how school violence is portrayed in the newspaper media. Articles discussing Columbine in *The New York Times* were analyzed for content for 30 days following April 20, 1999. Specifically, the research looked at the use of crime data, the explanation of cause for Columbine and other information included in the articles on Columbine. The research found that fewer than 10 percent of the 130 articles contained crime data leaving the reader with a very narrow view of school violence. Fifty-two percent of the articles identified a cause for the incident; of the articles with a cause, guns (gun control laws, availability of guns) were responsible in 31 percent of the articles. Logistic regression analysis showed that an article with a picture was 20.7 times more likely to appear on the front page than an article without any pictures ($p < .01$). The results suggest that the use of episodic framing dominated the articles on Columbine in *The New York Times*.

Introduction

Based solely on media reports, violence in schools has increased over the past twenty years. In reality, school violence has been decreasing, and most violence among youths occurs outside of school. During 2002, 88,000 students aged 12-18 were victims of violent crimes at school while 309,000 students of the same age were victims of violent crime outside of school. These numbers may seem shocking but they are relatively positive since victimization rates at school and away from school have declined between 1992 to 2001 (Bureau of Justice Statistics, 2005). The media sometimes engages in agenda setting- attracting attention to problems that they believe need to be addressed (Young, 2003). Thus, the misrepresentation of school violence in the media could be due to agenda setting. The current study examines how school violence is portrayed in the newspaper media. Specifically the coverage of the incident at Columbine is examined with articles printed in *The New York Times*. This study adds to the existing body of research focusing on school violence by examining how school violence is presented in the media.

Researchers have previously examined crime and its presentation by the news media. Erich Goode (2000, p. 549) believes that, "The mass media thrives on scares; contributing to moral panics is the media's stock in trade." The mass media has an impact on making people more fearful than they need be (Glassner, 1999). Heath (1984) proposed that crime stories are sensationalized because of their emphasis on crimes that are unusual and rare. For example, between July 1, 1999 and June 30, 2000, 16 school age children were involved in school related

homicides (Bureau of Justice Statistics, 2005). In that one year period, only one more student died in all the United States than those that died as a result of Columbine. Columbine was not the norm for violent incidents at schools across America, yet the media constantly displayed images from that day without mentioning the broader picture: school violence decreasing as a whole. Rather than focusing on isolated events that rarely occur, I am suggesting that writers use a more thematic approach (general context) when writing articles rather than the abundance of episodic framing (specific events) that pervades our newspapers today.

The ideas the media portrays (that violence is prevalent and more frequently occurring than in reality) direct social change and social movements. For this reason, the media should report news that is more representative of reality. One way to thematically frame articles in the newspaper is by incorporating actual crime statistics in articles that discuss school violence. The purpose of crime data is to provide contextual information surrounding school violence. However, this goal may be contrary to the desire of newspapers in attracting the largest number of readers/viewers and making the news interesting to all age groups.

In order to learn more about the way school violence is reported in the media, the current research will look at how the worst case of school violence in history is presented in one of our nation's top newspapers. School-violence-related articles from *The New York Times* will be analyzed for 30 days following the tragedy at Columbine (April 20, 1999) to determine whether school violence in the media is representative of school violence in reality. Several studies have examined the Columbine event, (Addington, 2003; Brener, Simon, Anderson, Barrios, & Small, 2002; Chyi & McCombs, 2004; Kostinsky, Bixler & Kettl, 2001) but none has looked at the use of crime data in reporting school violence in the newspaper. Crime data are reported in academic journals and government reports, but the media reaches the greatest number of people. If the media does not incorporate actual crime statistics in its reporting of school violence or violence of any sort, public perception of violence will be skewed. Biased public perception can affect the decisions politicians make whose primary concern is just to be re-elected.

Due to the importance of accurate reporting, I focused on the presence or absence of crime data in newspaper articles on the school violence which took place at Columbine High School. By means of content analysis, I examined how frequently crime data is used to report school violence in the newspaper. Also, I explored the speculation of cause by the newspaper media for the incident at Columbine, factors that influence the location of the article within the newspaper (front page or not), the focus of the articles discussing Columbine and the type of information included in the articles on Columbine. In addition to the content analysis, I observed the trends in school violence between 1992 and 2002 and compared that data with the number of school violence articles that appeared in *The New York Times* during the same time period.

Literature Review

Concerning the media's "hype" over school violence, Donohue, Schiraldi and Ziedenberg (1998) of the Justice Policy Institute investigated the reality of school violence and violence among youth outside of school. Specifically they asked: "Is there a trend towards increasing violent school deaths in America?" and "What is the overall incidence of crime, and particularly homicides, in America's schools versus outside of schools" (Donohue et al., 1998, p. 3)? Since school killings are recorded by several different sources using different criteria, Donohue et al. (1998) utilized data from multiple sources including the United States Department of Education, U.S. Department of Justice, Uniform Crime Reports from the FBI, Centers for Disease Control,

National Safe Kids Campaign, Office of Juvenile Justice and Delinquency Prevention, National School Safety Center, Center to Prevent Handgun Violence and the Bureau of Justice Statistics.

The National School Safety Center collected data annually since the 1992-93 school year to determine the number of deaths occurring in American schools over time; however, this survey measures “school related violent deaths,” which does not specifically define who is killing whom. An example of this measurement error is a homicide by an angry boyfriend who did not work at the school but shot a teacher in the school parking lot. The problem is that “school related violent deaths” include random acts of violence that may have been committed by someone other than a student. In 1997-98, four of the 40 “school related violent deaths” were adult deaths. Another drawback of the National School Safety Center’s survey lies in the fact that the Center relies on newspaper clippings to count school violence incidents, limiting the count to what the media chooses to cover.

Through data triangulation, Donohue et al. (1998) examined school violence in all its complexity. Data from the National School Safety Center suggests a 27% decline in school associated violent deaths from the 1992-93 school year to the 1997-98 school year.

From the Center for Disease Control’s study of “School-Associated Violent Deaths in the United States, 1992-1994” Donohue et al. (1998) reported less than one in a million chance of suffering a school associated violent death (homicides and suicides). Also, during the 1992-94 time period, more than 99% of violent deaths involving children occurred away from school.

The National Center for Education Statistics’ *Violence and Discipline Problems in U.S. Public Schools, 1996-1997* surveyed principals across America regarding the incidence of school violence. Only 10% of the principals within the sample reported the occurrence of serious violent crime, operationalized as murder, suicide, rape or sexual battery, robbery or physical attack with a weapon; none of the principals reported murder or suicide. Because school violence rarely occurs, we can not conclude from this small sample that no murder or suicide took place in all American schools during 1996-97. Broken down by school location, urban schools were more likely to report serious violent crime; 17% of urban schools, 11% of urban fringe schools, 8% of rural schools and 5% of suburban/town schools reported at least one incident of serious violent crime (Donohue et al., 1998).

Another finding that contradicts the media’s representation of crime is that 90% of all childhood deaths occur at places other than school. Data from the National Safe Kids Campaign show that unintentional shootings among kids occur more frequently when children are without adult supervision such as between 4 and 5 p.m. All of this media attention may affect the perceptions of politicians or they may receive pressure from their constituents to make changes to reduce school violence. Governor James Gilmore of Virginia actually proposed ending after school programs after a non-fatal shooting in Richmond despite research support for the effectiveness of after-school programs in reducing crime among youth (Donohue et al., 1998).

Focusing on the media’s representation of school violence, Chyi and McCombs (2004) examined coverage of the tragedy at Columbine in *The New York Times*. Their research analyzed the content of articles and editorials for 30 days following the event. Their content analysis involved a space and time frame measurement for each article. The space frame measured the focus of the article on five levels: *individual* if the story focused on individuals such as offenders and victims; *community* if the article focused on Columbine High School, Littleton or any other single community; *regional* if the story focused on Denver, Colorado, or any state or region; *societal* if nation-wide concerns were discussed; *international* if the story was related to events in other countries.

Of the 170 articles and editorials found in *The New York Times* for the 30 days following Columbine, Chyi and McCombs (2004) found that 52% of the stories had a societal frame, 29% had a community frame and 17% had an individual frame. Two percent used a regional frame and only one percent used an international frame. Furthermore, the researchers took note of the change of focus as the 30 days progressed. During the first 25 days, the percentage of societal frames gradually increased from 38% to 78%, while percentage of individual frame articles decreased from 30% to 0%. This finding demonstrates the shift in focus from specific information about the event and the people surrounding it to a broader examination of school violence as a social problem.

In addition to analyzing articles in *The New York Times*, Menifield, Rose, Homa and Cunningham (2001) compared media coverage of urban and rural school violence in the *Wall Street Journal*, *Louisville Courier Journal*, *New York Times*, *St. Louis Post Dispatch*, *Los Angeles Times*, and *Washington Post*. The purpose of their research was to determine if the newspaper media publish misleading views of school violence.

The content analysis of the six newspapers included the title of the article, the tone of the article, location within the newspaper, word count, lines, paragraphs and pictures used in the article. The *Courier Journal*, *Wall Street Journal*, and *New York Times* came from the library microfilm collection at Murray State University, Murray, Kentucky. The data from the *Washington Post*, *Los Angeles Times*, and *St. Louis Post Dispatch* were found in the microfilm collections at Southern Illinois University at Carbondale and Illinois State University at Bloomington-Normal. Three rural and three urban schools where shootings occurred were examined through the content analysis.

Menifield et al. (2001) found that the three rural school shootings received a disproportionate amount of attention compared to the three urban school shootings. The urban shootings received little attention from the six newspapers along with the number of pictures devoted to those shootings. For example, in the *Courier Journal*, photos pertaining to the rural school shootings totaled 101, while the total number of photos for the urban school shootings was two. The high amount of reporting on rural school violence may be due to the assumption that rural schools are safer than urban schools. The National Center for Education Statistics' *Violence and Discipline Problems in U.S. Public Schools, 1996-1997* surveyed principals across America regarding the incidence of school violence and found that 17% of urban schools, 11% of urban fringe schools, 8% of rural schools and 5% of suburban/town schools reported at least one incident of serious violent crime (Donohue et al., 1998).

Although the previous research has focused on the print media it is important that other forms of news media are examined. Dorfman, Woodruff, Chavez and Wallack (1997) examined how television news structures the perceptions of the public and the policies created regarding youth violence. Their research involved content analysis of 214 hours of local television news in California which resulted in 1791 stories concerning youth, violence or a combination of youth and violence. These stories were coded and analyzed to determine whether they had a public health perspective (more thematic rather than episodic and not blaming the individual for the problem).

Dorfman et al. (1997) found that violence dominated the news stories and the specifics of particular crimes dominated the violence coverage in the news. One-half of the youth stories included violence while two-thirds of the violence stories involved youth. Their major finding was that episodic coverage was five times more prevalent than thematic coverage which refers to the broader picture of youth violence.

Along with research done on school violence, researchers have also focused only on the Columbine school event. Similar to Menifield et al. (2001) interest in the media's portrayal of school violence, Addington (2003) focused solely on the effect Columbine had on the fear of victimization among 12-18 year-old U.S. primary and secondary school students. Using the school crime supplement (SCS) to the national crime victimization survey (NCVS) a random sample of 12-18 year old U.S. students was surveyed pre and post Columbine. The NCVS and SCS from 1995 and 1999 were used in the analysis. Students were interviewed from January to June of each year. The pre-Columbine group contained two-thirds (5620) of the 1999 sample of the NCVS-SCS while the post Columbine group contained one-third (2777) of the students interviewed. The respondents were randomly allocated to the two groups resulting in no systematic differences between the groups.

The SCS asks questions about student's fear of victimization, perceptions of school security measures and avoidance behaviors such as staying home from school. The two questions from the SCS regarding fear of victimization included, "How often are you afraid that someone will attack or harm you at school?" and a similar question, "How often are you afraid that someone will attack or harm you on the way to and from school?" Other questions from the SCS dealt with security at school and areas that students avoid at school because of fear that someone might harm or attack them.

Addington (2003) found that the students interviewed post-Columbine reported more fear at school than the students who were interviewed before Columbine. Of the 3.8% who reported more fear, only one-fourth of the students were afraid "sometimes" or "most of the time" while three-fourths were "almost never" afraid at school.

This study also examined school security before and after Columbine; however, only six security measures were assessed. Schools may have implemented other security programs after Columbine that were not included in the SCS. Also, the interviewing only lasted until June, a short period of time after Columbine when most schools may not have had the time to create and implement security measures.

Focusing again on the effects of Columbine, Kostinsky et al. (2001) sought to determine whether the abundance of threats following the incident at Columbine was catalyzed by imitation. Using a database of school violence threats reported to the Pennsylvania Emergency Management Agency (PEMA), Kostinsky et al. (2001) analyzed the amount of threats from different counties and school districts in Pennsylvania along with demographic data from the U.S. Bureau of Census county estimates.

Before 1999, school administrators estimated only one to two threats per year; however, during the 50 days following the Columbine incident, Pennsylvania schools reported 354 threats of school violence. The threats were made in a crescendo-decrescendo pattern. Fifty-six percent of the threats occurred on or before the tenth day after Columbine, and more than one-third of the threats were on days eight, nine and ten. Kostinsky et al. (2001) found the major predictors of the likelihood of violence to be a larger proportion of white than non-white students and a larger school enrollment.

The research done by Kostinsky et al. (2001) is instrumental in the prevention of future school violence and school threats. Their research stressed the importance of realizing that imitation threats can occur. The media should be aware of this and construct their stories with this fact in mind. The well-being of students and those who work with them should be valued more than attracting the most attention to a story. More importantly the students who commit these crimes should not be portrayed as heroes. "It would be more prudent following similar

events in the future to shift the focus from the countercultural aspects of the crime to the fact that the perpetrators are troubled individuals, who often suffer from psychiatric disorders, need help, and do not know where to get it” (Kostinsky et al., 2001, p. 1001). Thus it would be important to include the names and telephone numbers of organizations that provide help for troubled individuals at the end of or within the articles.

Brener et al. (2002) studied the effect of Columbine on reports of violent behavior and suicide among U.S. high school students. Specifically they examined whether students missed more school following Columbine due to feeling less safe as a result of the Columbine incident. Brener et al. (2002) also looked at weapon carrying after Columbine along with increased fighting and other violent behavior among students.

Using the 1999 Youth Risk Behavior Survey (YRBS), Brener et al. (2002) examined nationally representative data measuring behaviors related to violence and suicide. Students in grades nine through twelve were obtained through a three-stage cluster sampling. All public and private high schools in the U.S. were part of the target population. A total of 15,349 students completed questionnaires between March 1 and June 4 of 1999; however, 78.4% of the respondents completed the survey on or before April 20, 1999, the date that Columbine occurred.

The survey contained questions about weapon carrying, gun carrying, weapons carrying on school property and missing school because of feeling unsafe 30 days or less before the survey. There were five questions related to suicide and eight questions regarding interpersonal violence. Also included in the survey were questions about physical fighting on and off school property.

Brener et al. (2002) found that after Columbine, 10.2% of students reported feeling too unsafe to go to school compared to 3.9% of students before Columbine. Surprisingly, students completing the questionnaire after Columbine were less likely to report considering suicide or making a suicide plan than the students who completed the survey before April 20, 1999. The effects of geographic region on feeling too unsafe to go to school were most pronounced for rural areas more so than urban and suburban areas; however, their change was significant, also. For rural areas the percentage of students who felt unsafe increased from 3.8% to 32.6%.

Questions

Question 1: How often did The New York Times use actual crime statistics when reporting on Columbine during the 30 days following the event?

In the current research, it is expected that episodic coverage will dominate the Columbine stories. This will be shown by the lack of crime data used to report school violence. By excluding crime data, the reader will only have a limited view of school violence believing that it is more frequently occurring than it really is. This is consistent with the Dorfman et al. (1997) research which found that episodic coverage was five times more prevalent than thematic coverage in stories concerning youth and/or violence. Therefore, I believe that this research will find little crime data in newspaper articles on Columbine resulting in more episodic than thematic coverage in the articles.

To make news stories more interesting, journalists focus on sensational descriptions of the offenders and victims specific to the event being covered rather than including crime statistics that may not reflect the violence they are covering. By providing information on the broader picture (i.e. a decreasing crime rate) the story may not seem as interesting or capture the attention of such a large audience. At the time of Columbine, school violence was decreasing;

however, this fact probably will not be presented in many articles. More attention is drawn to articles by focusing on the peculiarities of an isolated event.

Question 2: How representative is the amount of newspaper coverage of school violence between 1992 and 2002 compared to the actual occurrence of school violence during the same time period?

I predict that the number of articles appearing in *The New York Times* each year will not be representative of school violence in reality. The results from the Menifield et al. (2001) study of media coverage of urban and rural school violence support this reasoning. Menifield et al. (2001) found that the three rural school shootings received a disproportionate amount of attention compared to the three urban school shootings despite the fact that urban school violence is more frequently occurring than rural school violence (Donohue et al., 1998).

Exploratory Question 1: What will the media most frequently speculate as the cause for the Columbine incident?

Due to the absence of literature regarding this topic I do not know what to expect the media to speculate as the cause for the Columbine incident. Based on the fact that past research has not found the reporting of violence to be representative of violence in reality (Dorfman et al. 1997; Menifield et al. 2001), I would not expect their speculations to be in-depth, discussing school violence as a social problem or the offenders as troubled individuals who need help.

Hypotheses

Hypothesis 1: A majority of the Columbine articles will have a societal frame rather than individual, community, regional or international.

I expect to have similar findings to Chyi and McCombs (2004) regarding the framing of the articles on Columbine. In their study of media salience and media coverage of Columbine, they found that 52% of the 170 stories they found in *The New York Times* had a societal frame, meaning that the articles focused on nation-wide interests. Because I am replicating this part of their project I expect to have similar findings; however, I am not going to include editorials although Chyi and McCombs (2004) did include editorials for their analysis.

Hypothesis 2: The front page articles will contain more graphic pictures and use episodic reporting and sensationalized words to describe Columbine more than non-front page articles in order to attract the most number of readers.

In their analysis of urban and rural school violence reporting in several newspapers including *The New York Times*, Menifield et al. (2001) found, “the newspapers sought to capture the attention of the audience with glaring headlines and then to elicit sympathy for those involved by running front-page pictures with intense captions” (p. 458). I believe that this research will have the same outcome as that of Menifield et al. (2001), especially because Columbine was the worst case of school violence in history.

Methods

Content Analysis

Content analysis was done using the New York Times Historical database. The articles were found by conducting an advanced search for “Columbine” OR “Littleton” in the citation or document text of articles for each of the 30 days following April 20, 1999. From April 21 to May 20, 1999, 130 articles appeared in *The New York Times* that discussed Columbine. Editorials and letters to the editor were excluded from the analysis due to their highly opinionated nature. Also, the basis on which these editorials and letters are selected is unknown; there are many more written than are published. Articles that did not directly discuss Columbine but mentioned the event (and otherwise would not have been written if it were not for Columbine) were analyzed in addition to the articles that directly focused on Columbine. Examples of these articles include discussion of the gun control debate, bomb threats at schools across the United States, bomb threats at schools across the United States or articles about the effects of violence in the media.

In order to gain rich descriptive information on school violence reporting in *The New York Times*, the articles were analyzed using the following criteria: date, location in paper (front page or not), headline, whether or not crime data were included and the type of data, focus of article (individual, community, regional, societal or international), words used to describe the Columbine incident, whether or not the offenders and/or victims of Columbine were discussed, number of words in article, number of pictures used and whether or not the article attempted to explain the cause of the event. The focus measure was taken from research done by Chyi and McCombs (2004). Chyi and McCombs (2004) established that an article with an individual focus concentrated on individuals such as the offenders, victims or their families. A community-oriented article focused on Littleton, Columbine High School or any other community across the nation. The article was coded as regional if it focused on Colorado, the Denver area or any other state or region. An article coded as societal discussed national interests, and lastly, international articles focused on events in other countries. This research deviated slightly from Chyi and McCombs’ (2004) criteria for community and regional focused articles. In this research, an article from a different area of the country was coded as regional even if the article focused on a community in another part of the United States; whereas, Chyi and McCombs (2004) chose to code it as community. Only articles that discussed Columbine or Littleton were coded as community in this research.

After the data were collected, the “explanation of cause” category was coded. If an article speculated the cause of the event, the response was then coded into one of five categories. The categories include: guns (weak gun laws, gun availability), cliques, violent media (video games, movies, music, internet), a combination of factors (could be the aforementioned causes or other factors) and lastly a category for “other” which included examples such as psychiatric disorders, suburban design or children not being screened for emotional instabilities. If an article focused on the gun debate (weak gun laws, etc.) while citing the incident at Columbine, it was implied that the speculation of cause was guns. Thus the article did not have to directly claim that weak gun laws were the cause of Columbine.

The number of pictures category ranged from 0 to 12 and was recoded into “having” or “not having” pictures because the specific number of pictures was not important. The descriptive words that were recorded for each article were coded based on the degree of seriousness. On a scale from one to ten, each article’s descriptive words were rated with ten being the most descriptive and inciting, and one being the least descriptive and inciting.

Furthermore the data was recoded into “0” if it had no descriptive words, “1” for words ranging from two to five on the original scale, and “2” for words ranging from six to ten on the scale. For example, words such as “bloody rampage” and “carnage” were coded as a ten, while words such as “jocks, preps and trench coat mafia” were coded as a two. In one of the articles, a quote from a classmate of one of the killers stated that Dylan Klebold was a “nut case.” Although the statement is quite derogatory, it was only coded as a three due to the varying degree of words used to describe the incident at Columbine.

The date was also recoded into three categories: “0” for the first ten days following Columbine (April 21-30, 1999), “1” for the next ten days (May 1-10, 1999) and “2” for the last ten days following Columbine (May 11-20, 1999).

Analysis of School Violence Trends

The other portion of this research compared the number of school violence related articles in *The New York Times* with the actual school violence rates for each year from 1992 to 2002. Data from the 2004 *Indicators of Crime and Safety* were used to compare school associated violent deaths with the actual number of school violence articles in *The New York Times*. The rate of nonfatal crimes (violent crimes and theft) among youth at school was also graphed and compared to the number of articles appearing in *The New York Times*. The following definitions were taken from the 2004 report.

School-associated violent deaths include a homicide, suicide, legal intervention, or unintentional firearm-related death in which the fatal injury occurred on the campus of a functioning elementary or secondary school in the United States, while the victim was on the way to or from regular sessions at such a school, or while the victim was attending or traveling to or from an official school-sponsored event. Victims included students, staff members, and other nonstudents (Table 1.1, “Indicator 1,” n.d.).

Serious violent crimes include rape, sexual assault, robbery, and aggravated assault. Violent crimes include serious violent crimes and simple assault. Total crimes include violent crimes and theft. “At school” includes inside the school building, on school property, or on the way to or from school (Table 2.2, “Indicator 2,” n.d.).

The purpose of this part of the investigation was to determine how representative newspaper reporting is of actual school violence rates. The search for articles may not have produced all the articles on school violence each year, but the search was consistent from year to year. Using the New York Times Historical database, articles were generated by searching for “school violence” in the document text of articles from January 1 to December 31 of each year. Due to the limited nature of the search engine, front page articles were not included in the search for articles. This among other search restrictions did not allow for a full list of articles on school violence related events. For example, I found 130 articles on Columbine in one month of 1999, but the latter search only produced 56 articles using “school violence” as the keyword. Consequently, articles that discussed school violence events but did not contain the words “school violence” in the document text were not included in the count.

Results

Content Analysis

Only 11 (8.5%) of the 130 articles contained crime data. The following crime data were founding the 11 articles.

Data used in reporting Columbine in The New York Times

1. 1997 Study by Journal of American Medical Association: states that enacted laws making parents liable if they don't store guns properly saw 23% decrease in number of accidental shootings of children
2. Bureau of ATF: 0.1% of dealers supplied 13,000 guns in crime in 1998
3. Center for Disease Control and Prevention: less than 1% of child homicides occur in or around schools
4. FBI: crime fell 7%; Dr. James Alan Fox: "school is safest place to be"
5. Michael Moore, attorney general of Mississippi: 12 children die a day
6. National School Safety Center in CA: school violence is rare and declining, less than 1% of schools in US had death on campus in last 7 yrs
7. National School Safety Center of Westlake Village, CA: school violence down from 5 years ago.
8. Pennsylvania Emergency Management Agency: 19 bomb threats in 17 counties following Columbine
9. Research on 120 adolescent suicides; 90% had diagnosable psychiatric disorders
10. Research studies cited for finding some effects of media violence on children's behavior
11. Surgeon General Dr. David Satcher: 13 young people die due to violence each day

Although 11 out of the 130 articles used crime data to report school violence in the 30 days following Columbine, lines #5 and #11 above are very ambiguously stated. Do 12 children die a day in school? Due to the fact that this statement was reported in an article on school violence, it is not unreasonable to assume that some readers would believe that many of these violent deaths occur at school. The statement, "12 (or 13) young people die due to violence each day" does not fully describe adolescent violence. In fact, this line of thinking would be erroneous because statistical data provides evidence that, "Students are more apt to be victims of serious violent crime away from school than at or on the way to school" (School Crime Victimization, "Juveniles as Victims," n.d.).

Of the 130 articles found in *The New York Times* that covered Columbine, 18.5% of the articles were on the front page. On average, almost one article was printed per day on the front page of *The New York Times* following Columbine. For the 30 days following the incident, 24 (18.5%) of the articles were front page stories.

Using the criteria from Chyi and McCombs (2004) study of media salience and news coverage of Columbine, I found 16.2% of the articles contained an individual focus, 13.1% a community focus, 21.5% a regional focus, 46.9% a societal focus and 2.3% an international focus.

The next research question concerns what the article speculated as the cause for Columbine. Overall, 52% of the articles speculated a cause for the incident. Guns were the cause in 30.9% of the articles that speculated a cause. A combination of factors was discussed as the cause 27.9% of the time, while cliques and violent media were responsible 11.8% and 10.3% of the time, respectively. The "other" category yielded 19.2% of the explanations for Columbine.

The other category included such explanations as psychiatric disorders, suburban design or children not being screened for emotional instabilities.

Information regarding the offenders at Columbine along with the victims from the incident appeared at about the same rate in the articles. Twenty percent of the articles contained offender information and 21.5% contained information about the victims. Examples of offender or victim information include demographics, opinions of the offenders from anyone who knew them or reactions from victims and families to the Columbine incident.

The number of pictures for each article was also recorded during analysis. Almost 42% (54) of the articles had zero pictures, while the remaining articles had at least one picture. Forty percent (52) of the articles had one picture, 13.1% (17) had two, 2.3% (3) had three, 0.8% (1) had four, 1.5% (2) had five and 0.8% had 12 pictures. In addition to the number of pictures, a word count was taken from the New York Times Historical Database. The minimum number of words was 102, while the maximum was 3967. The mean was approximately 993 with a standard deviation of 530.

Table 1 presents the correlations of variables used in the logistic regression analysis. At the 0.05 level of statistical significance, speculation of cause and description of the incident were significantly correlated with an article being on the front page. Word count, offender information, victim information and whether or not there was a picture were correlated with front page articles at the 0.01 level of significance. Offender information and description of the incident were significantly correlated with the date ($p < .05$), while victim information was significantly correlated with the date at the 0.01 level. Word count was significantly correlated with speculation of cause ($p < .01$). Offender information, victim information and the picture variable were significantly correlated with word count at the 0.01 level. The picture variable ($p < .05$), victim information ($p < .01$) and description of the incident ($p < .01$) were significantly correlated with offender information. The picture variable was significantly correlated to victim information at the 0.05 level; the description of the incident, at the 0.01 level. Lastly, whether or not crime data were presented was significantly correlated with the picture variable at the 0.05 level.

Table 2 presents the logistic regression with front page (0=no, 1=yes) as the dependent variable. At the 0.01 level of significance an article with a picture was approximately 20.7 times more likely to appear on the front page than an article with no pictures. An article that did speculate a cause was 3.6 times more likely to appear on the front page than an article that did not ($p < .05$). Articles containing victim information were 5.7 times more likely to appear on the front page than articles that did not contain information on the victims of Columbine and was also significant at the 0.05 level. Because of the relatively small number of cases ($N=130$) I chose to include relationships significant at the 0.1 level: articles containing offender information were 3.7 times more likely to be located on the front page than articles that did not contain offender information. At the same level of significance, articles on the second ten days following Columbine (May 1-10) were 1.2 times less likely to be on the front page than articles written in the first ten days following the event.

Table 1
Correlation matrix with variables in the logistic regression model, N=130

	Mean	Std. Dev.	1	2	3	4	5	6	7	8	9
(1) On front page	0.18	0.39	1								
(2) Date	0.65	0.77	0.12	1.00							
(3) Speculation of Cause; 0=none, 1=yes	0.52	0.50	0.18 *	0.02	1.00						
(4) Word Count	992.56	529.92	0.42 **	0.09	0.32 **	1.00					
(5) Offender information; 0=none, 1=yes	0.20	0.40	0.36 **	0.22 *	0.02	0.26 **	1.00				
(6) Victim Information; 0=none, 1=yes	0.22	0.41	0.38 **	0.32 **	0.10	0.34 **	0.49 **	1.00			
(7) Picture; 0=none, 1=yes	0.58	0.49	0.36 **	0.08	0.10	0.51 **	0.19 *	0.18 *	1.00		
(8) Description of incident; 0=no desc, 1=less desc, 2=more	0.30	0.64	0.18 *	0.18 *	0.06	0.13	0.31 **	0.28 **	0.13	1.00	
(9) Crime data present; 0=no; 1=yes	0.08	0.28	0.07	0.00	0.07	0.05	0.15	0.16	0.19 *	0.03	1

* significant at the .05 level
**significant at the .01 level

Table 2

Logistic Regression with Front Page (0=no, 1=yes) as the dependent variable, N=130

Variable Name	B		Exp(B)
Date			
First 10 days (April 21-April 30) (reference group)		*	
Next 10 days (May 1-10)	-1.23769	*	0.290054
Last 10 days (May11-20)	0.534172		1.706035
Speculation of Cause; 0=none, 1=yes	1.288139	**	3.626033
Word count	0.000927		1.000927
Offender information; 0=none, 1=yes	1.306661	*	3.69382
Victim Information; 0=none, 1=yes	1.746094	**	5.732168
Picture; 0=none, 1=yes	3.029402	***	20.68485
Description of incident			
No description (reference group)			
Less descriptive	-0.3774		0.685639
More descriptive	-0.04025		0.960552
Crime data present; 0=no; 1=yes	1.391732		4.021809
Constant	-6.54179		0.001442
* significant at the 0.1 level			
**significant at the 0.05 level			
***significant at the 0.01 level			

Analysis of School Violence Trends

The second portion of the research involved comparing the number of articles on school violence in *The New York Times* with the actual amount of school associated violent deaths per year from 1992 to 2002. Nonfatal crimes against students from 1992-2002 were also compared with the number of school violence articles in *The New York Times*. Figure 1 shows the amount of school associated violent deaths per year with data from the *2004 Indicators of School Crime and Safety* conducted by the National Center for Education Statistics (NCES) (Table 1.1, "Indicator 1," n.d.). Figure 2 is a graph of nonfatal crimes against students age 12-18 per 1000 students (Table 2.2, "Indicator 2," n.d.). Figure 3 shows the number of articles appearing in *The New York Times* when searching for the words "school violence" in the document text of articles each year using the New York Times Historical Database.

When comparing the data from the three graphs, the number of articles on school violence in *The New York Times* is not representative of the actual occurrence of school associated violent deaths or nonfatal crimes against students. The peak number of articles on school violence appeared in 1999, the year of Columbine; however, school associated violent deaths and nonfatal crimes were low in 1999 relative to previous years. Also, take note that the number of nonfatal crimes greatly exceeds the number of school associated violent deaths in any year. Less serious crimes are more frequently occurring, yet it appears the media chooses to report the least frequently occurring incidents such as Columbine.

The results of this comparison of articles in the newspaper with actual crime data displays the randomness of reporting by the newspaper media. During the years when crime was higher in schools, the amount of reporting was down. The amount of articles on school violence clearly does not represent school violence in reality. In future examination of this comparison it would

be beneficial to use more than one newspaper from different areas of the country to get a more representative sample of articles on school violence. These findings are only preliminary.

Figure 1
School Associated Violent Deaths per Year, 1992-2002

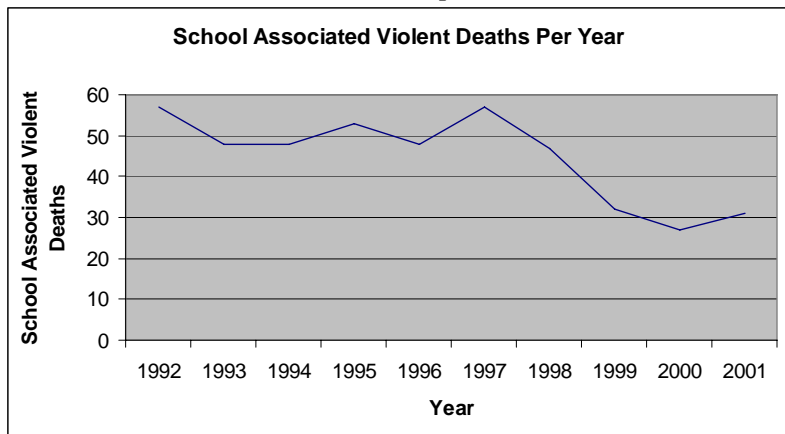


Figure 2
Nonfatal Crime for Students Age 12-18 per 1000 Students, 1992-2002

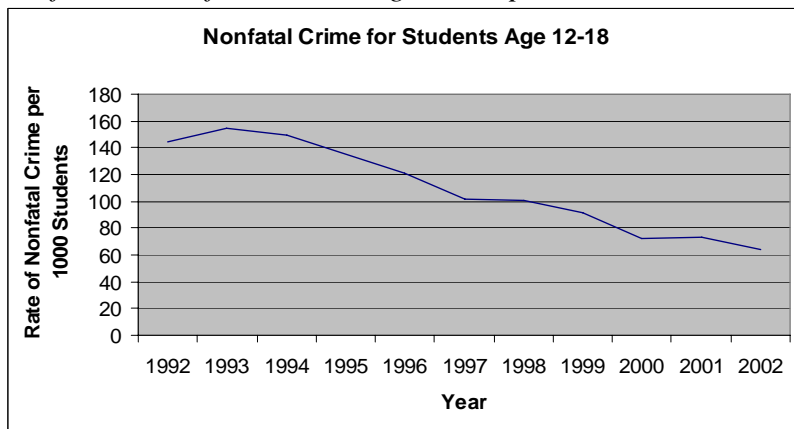
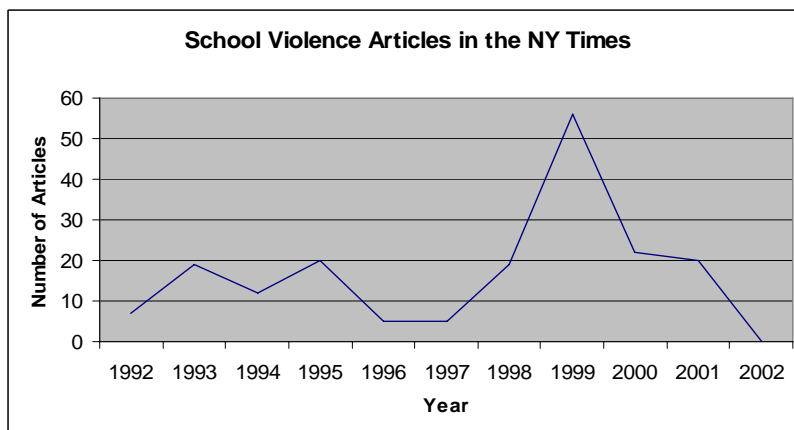


Figure 3
School Violence Articles in The New York Times, 1992-2002



Discussion and Conclusion

From this study we can conclude that *The New York Times* did not frequently report crime data in articles that covered Columbine for 30 days following the incident. Eleven of the 130 articles contained crime data and only one of the front page articles used crime data to report on Columbine. Just as Dorfman et al. (1997) found that episodic coverage was five times more prevalent than thematic coverage, the lack of crime data found in this study suggests similar findings. Incorporating crime data in articles on school violence provides a broader picture and allows the reader to better understand social issues such as school violence. In addition to the findings on crime data, more individual and community focused articles were on the front page (as opposed to not being on the front page) than regional, societal or international articles. Focusing on individuals or the community of Columbine or Littleton does not help to provide a broader view of school violence.

Besides the lack of crime data included in articles on school violence, it is also important to determine whether or not the reporting of school violence is representative of the actual rates of school violence. Donohue et al. (1998) sought to find the truth regarding school violence due to all the media hype over the issue; still, their research did not compare actual school violence with school violence in the media. In order to understand whether or not there is media hype over school violence, one must compare what is occurring in reality with what is reported. Menifield et al. (2001) compared the newspaper coverage of three rural school shootings and three urban school shootings using more than one newspaper for analysis which provided a more representative sample of the articles on the six school shootings they examined. They found more attention brought to the rural school violence incidents than to the urban school violence incidents. Although Menifield et al. (2001) research looks at specific characteristics of school violence (urban vs. rural) and this research takes a broader look at school violence, their results concur in part with the findings of this study that school violence in the media is not representative of school violence in reality. By comparing Figures 1, 2 and 3 from the data on school violence trends, school violence articles in *The New York Times* are not representative of school associated violent deaths or nonfatal crimes between 1992 and 2002.

The focus of the articles on school violence is important due to the effect the focus of an article may have on public perception. The criteria for this analysis of the framing of the article were based on research done by Chyi and McCombs (2004). In their examination of school violence articles in *The New York Times*, Chyi and McCombs (2004) based their findings on 170 articles including editorials. They found 17% had an individual frame, 29% had a community frame, 2% had a regional frame, 52% a societal frame and only 1% an international frame. The main discrepancy between this research and previous research done by Chyi and McCombs (2004) was with regard to the community and regional frames. Chyi and McCombs (2004) found 29% of the articles having a community frame while this research found only 13.1% with a community focus. Chyi and McCombs (2004) found only 2% of the articles to have a regional frame while this research, 21.5%. The coding for community and regional focused articles was different in this research than in Chyi's and McCombs' (2004) research which would explain part of the difference in results for these two categories. Another explanation is due to the fact that Chyi and McCombs (2004) chose to include editorials in their analysis which could have accounted for some of the community articles they found.

According to the results of this study and previous research, Americans may be misled to believe that school violence is more frequently occurring than it really is. The lack of crime data

and the strong focus on isolated events do not provide an accurate view of school violence. Regarding school violence,

Americans are not just misinformed, but are in many cases exponentially misinformed, by the hyperbole that too often follows school shootings. Americans don't know what they know about youth violence from personal experience, they know what they know about youth violence from the media. Most Americans report they get most of their information about violence from television (DeJong, 1999). As it turns out, the media has been a very poor teacher (Brooks, Schiraldi, & Ziedenberg, 2000).

Through this research, I wanted to examine actual school violence as compared to the media presentation of school violence. This provides a clearer picture of the real problems that adolescents face. By focusing all of our attention on isolated incidents such as Columbine, we divert energy from more important problems that teenagers face such as binge drinking, traffic crashes and other accidents which in total account for 42% of teenage deaths, explains Dr. John D. Graham, director of the Center for Risk Analysis at the Harvard University School of Public Health (Stolberg, Washington, 1999).

Limitations to this Research

In order to gain a broader understanding of school violence in the media, further research should examine more than one incident of school violence in more than one source of news media. More importantly, the television news media should be examined because it reaches more people than print news and there is probably more of an effect on public perception when one can see and hear the news. In addition to examining school violence in multiple sources of news media, it would be important to know what types of policies have been made regarding school violence and how effective they have been. It would also be reassuring to know that policies have been implemented based on real crime data and not simply on the basis of the coverage of isolated school incidents.

Implications: All the news that's fit to print?

Is anyone responsible for the way newspapers report school violence? It is easy to point the finger at the media; but they are only part of the problem. Newspapers should realize the effects of their reporting whether it results in more school violence, ineffective policies or copycat threats as described in the research by Kostinsky et al. (2001). Students may also be affected as Brener et al. (2002) and Addington (2003) found that students may be more afraid of victimization or attending school following the reporting of school violence incidents such as Columbine.

The media is not solely responsible for the effects of school violence in the media. The general public should cautiously review the information they receive from the media. If we become intolerable to the type of reporting that pervades the articles on school violence, the news media will have to change their reporting style to attract readers/viewers who have become more demanding for news that is more representative. Aside from the average American, policy makers should be aware of the misrepresentation of school violence in the media. However, even if they are mindful of the misrepresentation of violence in the media, the pressure from citizens to be tough on crime may induce politicians to strive only to satisfy their constituents even if that means inefficiently dealing with crime. For example, Governor James Gilmore (R) from Virginia proposed reducing the amount of nighttime athletic events to prevent an increase

in violence despite the fact that numerous criminologists have advocated the importance of after school programs for creating a safe environment for children (Donohue et al. 1998).

So, are we getting the whole picture regarding school violence? According to this study and previous research, we are not being fully informed. The incorporation of crime data in more articles along with more thematic coverage on school violence would help provide a better understanding of the problem we face regarding adolescent violence. We can't control the type of reporting the media uses to discuss school violence because that would infringe on their right to free speech; however, as a responsible organization, they can choose to report news in a morally acceptable way knowing that the effects of their reporting can be costly.

...Our society must address the problem of crime and violence in an informed and rational manner, making decisions based on documented empirical evidence rather than on distorted media images. Strategies and policies that are clearly ineffective and self-defeating must be discarded. Obviously the first step...is to educate ourselves about the reality, not the rhetoric, of the problems of crime and violence (Alvarez & Bachman, 2003, p. 10).

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A Biological Production of ZnS (Sphalerite) Nanoparticles with Desulfovibrio gigas

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Abstract

Sulfur-/sulfate-reducing bacteria (SRB) have been used for remediation of metal-contaminated ground water and soil through the precipitation of metal sulfides. These metal sulfides range from 3 nm to 3 μ m in diameter. Of specific interest to this research are zinc sulfide nanoparticles (ZnS NPs) produced by SRBs with a diameter of \sim 3 nm. These ZnS NPs can be used as catalysts to grow nano-wires, and these nano-wires, coupled with green fluorescent protein (GFP), might be used to make a cost-efficient biosensor. Currently, there is no report of a pure culture that produces ZnS NPs; therefore, this study investigates the ability of a pure culture of *Desulfovibrio gigas* in two different media (Media 63 and Media 149) to produce ZnS NPs as a metal precipitate. This study shows that both media are able to grow *D. gigas* in the absence of zinc in the solution. *D. gigas* growth in media containing zinc could not be determined due to ZnOH precipitates.

Introduction

Sulfur/sulfur reducing bacteria (SRB) are a family of bacteria that can take in soluble metals and produce insoluble metal precipitates. Many researchers have examined bacterial systems because they are relatively simple systems. SRB have been used in the remediation of flooded zinc mines in Tennyson, Wisconsin (Labrenz et al., 2000.) Researchers have exploited the anaerobic nature of the SRB to filter out the contaminated soils and water. Specific to this research, *Desulfovibrio gigas* is an obligate anaerobe that produces hydrogen sulfide as a by-product of energy production. Instead of using oxygen as the terminal electron acceptor *D. gigas* uses sulfate. The sulfate in return reacts with hydrogen and forms H₂S. H₂S in solution is highly reactive with many metal ions and forms an insoluble metal precipitate. These precipitates can be filtered out and hence remediate the water contaminated by the metal ions (Labrenz and Banfield, 2004.) This research looks at exploring this process to acquire the insoluble metal precipitates and use it as a catalyst to make nano-wires. This research explores allowing zinc ions to react with the H₂S and forming ZnS nanoparticles (NPs). These ZnS NPs will in turn be used as a catalyst to make ZnO nano-wires. In contrast, other studies have used a mix culture of SRBs to produce ZnS NPs, but this study examines the feasibility of using a pure culture of *D. gigas* to produce ZnS NPs.

Materials and Methods

D. gigas was acquired from Christopher House, Assistant Professor in Earth and Mineral Department at the Pennsylvania State University. The stock culture provided by Christopher House was grown in media 149. It was white and cloudy with bacterial growth and contained tiny black particles. Under normal conditions, the bacteria would be incubated for 4 days and sub-cultured once a week. For this study, *D. gigas* was stored in the refrigerator until the media for ZnS NPs were ready.

Growth media (Media 149) for *D. gigas* was obtained from the DSMZ.de culture collection website (<http://DSMZ.de>) which contains trace minerals, vitamins, and various sulfates. For the purpose of this work, a few changes were made to the formulation of this media. Since this media is only a growth media for *D. gigas*, it did not contain a zinc source for the ZnS nanoparticles, therefore, ZnSO₄ were added to the media. A base medium containing common ingredients for all the samples was prepared in a 500-mL media bottle which was capped with a rubber septum and a cap with a hole, about ½ in diameter, drilled in the center. The medium was degassed with N₂ gas in the fume hood for 15 minutes. A 1.5-in needle was placed into the hole in the cap of the media bottle to feed in the N₂ gas and another needle was inserted adjacent to the previous needle to allow excess gas to vent out. The medium was dispensed in five 100-mL serum bottles and capped with a rubber septum and sealed with an aluminum seal in an anaerobic chamber. Varying concentrations of ZnSO₄ were dispensed into the five media bottle; they were Zn-free (Sample A), 0.005g/mL (Sample B), 0.002g/ml (Sample C), 0.001g/ml (Sample D), and 0.01g/ml (Sample E.) These concentrations were arbitrarily picked to find a approximate level of zinc that is toxic to the bacteria. After the addition of zinc, they were subsequently removed from the anaerobic chamber and placed in a fume hood. In the fume hood, 1.5-in needles were placed into the serum bottle through the rubber septum, and they were flushed using 20% N₂ gas and 80% CO₂ gas followed by pulling a vacuum for three cycles. The media were autoclaved for 20 minutes in liquid cycle at 121 oC.

In contrast, another media formulation (Media 63) was obtained from the DSMZ website. This media was used to grow a mixed culture of SRB to produce ZnS NPs. This medium was prepared using the same technique to make Media 149. The only difference between the two media was the ingredients. Media 63 did not contain vitamin solutions and trace mineral solutions added to Media 149, so the same concentration used to make Media 149 was used. Five different samples with varying concentration of ZnSO₄ were made. They were Zn-free (Sample 1), 0.005g/mL (Sample 2), 0.002g/ml (Sample 3), 0.001g/ml (Sample 4), and 0.01g/ml (Sample 5.)

All ten media were inoculated with 2 mL of *D. gigas* and allowed to incubate at 30°C for 4 days. The inoculation syringe needle was flamed 4 times and inserted immediately into a 100-mL serum bottle which contained a stock N₂ gas. The N₂ gas was used to create an anaerobic atmosphere in the syringe. The needle was flamed 3 times and inserted into the stock culture of *D. gigas* in a 100-mL serum bottle and 2 mL of *D. gigas* was transferred to the fresh media.

The medium was dispensed in five 100-mL serum bottles in an anaerobic chamber. The five 100-mL serum bottles were flushed using 20% N₂ gas and 80% CO₂ gas and vacuumed for three cycles. Five media with varying concentration of ZnSO₄ were made; Zn-free (Sample A), 0.005g/mL (Sample B), 0.002g/ml (Sample C), 0.001g/ml (Sample D), and 0.01g/ml (Sample E). The media were autoclaved after adding the ZnSO₄. These concentrations were used to test for the zinc concentration that is toxic to the bacteria.

Another media formulation (Media 63) was obtained from the DSMZ website. This media was shown to be able to grow a mixed culture of SRB and produce ZnS NPs. The medium was dispensed into five 100-mL serum bottles in an anaerobic chamber. The five 100-mL serum bottles were flushed three times for 3 minutes using 20% N₂ gas and 80% CO₂ gas with a vacuum being applied in between flushing. For this media a varying concentration of ZnSO₄ was added; Zn-free (Sample 1), 0.005g/mL (Sample 2), 0.002g/ml (Sample 3), 0.001g/ml (Sample 4), and 0.01g/ml (Sample 5). In addition to the ZnSO₄, the vitamin solution and trace mineral solution used in Media 149 were added in an equivalent amount.

All ten media were inoculated with 2 mL of *D. gigas* and allowed to incubate at 30°C for 4 days. The needle of a sterile syringe were flamed and inserted into the stock N₂ gas to create an anaerobic atmosphere in the syringe. The needle is flamed again and inserted into the stock solution of *D. gigas* and 2 mL of *D. gigas* is transferred to the media previously made.

Results and Discussion

Media 63

After the media was made Zn(OH)₂ precipitate formed in the media. A simple test of combining different media components showed that zinc reacts with hydroxide ions in the solution to form Zn(OH)₂ at above pH 6. This caused a potential problem to this research because the normal growth media for *D. gigas* required a pH of 7.8. The media was prepared regardless of the problem and the bacteria were inoculated. Media 63 had a pH of about 6.3 and was slightly pinkish in color before inoculation. Some of the samples containing zinc had traces of precipitate. Five days after inoculation, a significant amount of bacterial growth was observed at the bottom of the serum bottles. The bacterial growth was viscous and looked gelatinous in texture; apparently growing as a biofilm as it resembled a membrane. Upon shaking the bottle several times, the membrane-like structure tore off from the bottom rather than it being re-suspended. Sample 1 had the most noticeable growth of this membrane like structure. In addition, Sample 1 had the most bacterial growth. Growth in Sample 2-5 could not be visually affirmed due to the presence of precipitate prior to inoculation. Bacterial growth of Media 63 containing zinc ions could not be determined. Further study is needed in using media with a lower pH to control the formation of Zn(OH)₂ precipitates.

It was anticipated that Media 63 might not support *D. gigas* growth due to the slightly acidic environment. The optimal growth pH for *D. gigas* was expected to be around 7.8 based upon the media formulation acquired from DSMZ. When pH levels are outside the limited tolerable range, certain membrane-bound proteins may become denatured due to disruption of ionic interactions among the amino acids, leading to loss of protein function. However, *D. gigas* was shown to be more resilient than expected and appeared to grow significantly in this media despite initial acidic conditions. This is fortunate since the low pH is useful to control precipitation. Unfortunately, the pH was not monitored; therefore, it is not possible to know if *D. gigas* was able to grow in the media because it was able to alter its environment, or because the pH conditions for growth are versatile. It would be interesting to test in the future the initial pH range in which *D. gigas* can grow and monitor how that pH changes during subsequent growth.

Media 149

Prior to inoculation, Media 149 had a pH of about 7.8 and it was colorless but cloudy with zinc hydroxide precipitates except for Sample A. After five days of incubation, Sample A was no longer clear; it became cloudy with *D. gigas* growth. Growth of the other samples could not be determined due to the presence of the precipitate. The precipitate was determined to be zinc hydroxide by testing various media components for precipitate formation. A solution of zinc ions was observed to react with other media components and produce a precipitate.

In concluding this summer research, both media type showed that *D. gigas* growth was indeed possible in the absence of zinc ions. Further research is needed in using media with a more acidic pH to control precipitation. In addition, based on the premise that *D. gigas* did grow in media with zinc ions, this experiment should precede to the next step of growing ZnO nano-wires.

Literature Cited

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