

The Penn State McNair Journal

Summer 2004, Volume 11

The Penn State McNair Journal is published annually and is the official publication of the Ronald E. McNair Scholars Program at Penn State. No responsibility for the views expressed by the authors in this journal is assumed by the editors or the Ronald E. McNair Scholars Program.

Copyright 2005 by the Ronald E. McNair Scholars Program at Penn State, all rights reserved.

The program is funded by a \$291,925 Ronald E. McNair Post-Baccalaureate Achievement Program grant from the U.S. Department of Education.

THE PENN STATE MCNAIR JOURNAL

Summer 2004

Volume Eleven

Table of Contents

Welcome from the Penn State McNair Scholars Program..... iii

TRIO Programs on the National Level.....iv

TRIO Programs at Penn State.....iv

McNair Scholars Program at Penn State.....v

McNair Scholars Summer 2004 Scholars and Staff.....vi

About Ronald E. McNair.....vii

McNair Alumni On The Move.....viii

McNair Scholar Articles

Interactions Between *Bordetella Bronchiseptica* and its Host
Luis Agosto..... 1

Towards Probing Skin Cancer using Endogenous Melanin Fluorescence
Andra Colbert..... 8

Assessing Parental Beliefs in Early Childhood
Trinity Crosby 16

Biomechanical Evaluation of Women Standing in High-Heeled Shoes
Paula Henderson..... 25

Dispersal Patterns of Male White-tailed Deer in Centre County, PA
Milton Newberry III 39

The Role of PPAR- α in Regulating Symptoms of Allergic Asthma
Tiffany Polanco..... 48

Biomechanical Evaluation of Crutch Design Variations
Adriana Segura..... 55

WELCOME

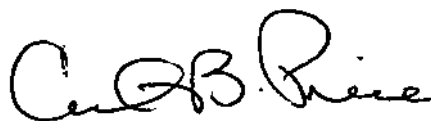
Since 1991, the Penn State McNair Scholars Program has enriched the lives of students at both Penn State and Virginia State University, our partner in the McNair collaboration. The McNair Program holds a very special place in our lives, as well as in the lives of the faculty and staff who work with our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

This is the eleventh issue of the Penn State McNair Journal. We congratulate the 2004 Penn State McNair Scholars and their faculty research advisors! This journal presents the research conducted in the summer of 2004 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 seven of the nine TRIO programs. There are two Educational Opportunity Centers in Philadelphia and 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 122 students earn their baccalaureate degrees. Of these graduates, 77 or 63 percent have gone on to graduate school at institutions across the country. Of this group, 15 or 19 percent have earned their doctoral or professional degrees and another 37 or 49 percent have earned their master's degrees. Currently, there are 37 or 49 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 our alumni have attended or now attend are: Penn State, Harvard, University of Cal-Berkeley, Stanford, Cornell, UCLA, Boston University, Indiana University, University of North Carolina-Chapel Hill, University of Maryland-College Park, University of Pennsylvania, and Ohio State University.

Summer 2004 McNair Scholars and Program Staff



Standing (left to right): Luis Agosto, Milton Newberry III, Jamal O'Garro, Curtis Price (Academic Coordinator)

Sitting (left to right): Teresa Tassotti (Program Director), Andra Colbert, Trinaty Crosby, Lori Jellick (Summer Program Assistant), Adriana Segura, Tiffany Polanco, Paula Henderson, Judy Banker (Staff Assistant)

Missing from the photo- Kenya Ramey

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)

PROGRAM AND EDITORIAL STAFF

Teresa Tassotti, Director

Curtis Price, Academic Coordinator

Judy Banker, Staff Assistant

RESEARCH METHODS COURSE INSTRUCTOR

Edgar Farmer

FACULTY RESEARCH ADVISORS AND EDITORS

Avery August

Duane Diefenbach

Eric Harvill

Ahmed Heikal

Stephen Piazza

Douglas Teti

SOCIAL MENTORS

Paul Alkebulan

Vernon Carraway

Danielle Downs

Ralph Gatrone

Eric Harvill

Joyce Hopson-King

Nelson Marquina

Philip Mohr

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 and 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
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
	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
Natasha Deer (PSU 1995)	M.A., Florida State University
Alicia DeFrancesco (PSU 1997)	M.B.A., Babson College
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
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
Leshawn Kee (VSU 1998)	M.A., Regents University
Haroon Kharem (PSU 1996)	Ph.D., Penn State
Carrie (Hippchen) Kuhn (PSU 2001)	M.A., Stanford University
Judy Liu (PSU 1995)	Ph.D., University of California-Berkeley
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
Robert Osmanski (PSU 1995)	M.S., Penn State
Lilliam Santiago-Quinones (PSU 1998)	M.Ed., Bowling Green State University
Thomas Shields (PSU 1998)	M.A., Penn State
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
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
Ndidi Moses (PSU 2000)	M.A., Penn State, now pursuing J.D. at University of Connecticut
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...

Felix Acon-Chen (PSU) May 2004
 Christopher Arlene (PSU) August 2004
 Heneryatta Ballah (PSU) May 2004
 Omotayo Banjo (PSU) May 2004
 Melanie Bombar (PSU) December 2004
 Jorge Delgado (PSU) May 2004
 Charmayne Maddox (PSU) May 2004
 Edward Mills (VSU) December 2004
 LaShauna Myers (PSU) December 2004
 Kristin Rauch (PSU) May 2004
 Luisa Soaterna (VSU) May 2004
 Kashra Taliaferro (PSU) May 2004
 Selima Taylor (PSU) May 2004
 Anthony Paul Trace (PSU) May 2004

On to graduate school in Fall 2004...

Felix Acon-Chen (PSU 2004) is pursuing graduate studies at Stevens Institute of Technology.
 Heneryatta Ballah (PSU 2004) is pursuing graduate studies at Ohio State University.
 Omotayo Banjo (PSU 2004) is pursuing graduate studies in Media Studies at Penn State.
 Jorge Delgado (PSU 2004) is pursuing graduate studies in Aeronautical Engineering at Purdue University.
 Charmayne Maddox (PSU 2004) is pursuing graduate studies in Education at Penn State.
 Edward Mills (VSU 2003) is pursuing graduate studies in History at University of Illinois at Urbana-Champaign.
 Kristin Rauch (PSU 2004) is pursuing graduate studies in Sociology at University of California-Davis.
 Luisa Soaterna (VSU) May 2004 is pursuing graduate studies in Physics at John Hopkins University.

In graduate school as of Fall 2004...

Juan Abreu (PSU 2002)	Rutgers University (Law/Criminal Justice)
Taimarie Adams (PSU 2003)	Harvard University (Law/Social Work)
Catherine Crawford (PSU 1996)	Central Michigan University (Education)
Lurie Daniels (PSU 2000)	New York University (Law)
Max Fontus (PSU 1999)	Indiana University-Blomington (Chemistry)
Tiana Garrett (VSU 2001)	University of North Carolina-Chapel Hill (Chemistry)
Michael Godeny (PSU 2002)	University of Florida (Physiology)
Sherie Graham (PSU 2002)	University of Michigan (Public Health)
Dustin Holloway (PSU 2002)	Boston University (Molecular Biology)
Mimi (Abel) Hughes (PSU 2002)	University of Southern California (Atmospheric Sciences)
LaShawne (Long) Miles (PSU 2001)	Xavier University (Education)

Lourdes Marcano (PSU 1995)
Leanna Mellott (PSU 2000)
Angel Miles (PSU 2003)
Kizzy (Frey) Nicholas (PSU 2000)
Tracie Parker (VSU 2003)
Cavin Robinson (PSU 2002)
Caryn Rodgers (PSU 2000)
Kahlil Williams (PSU 2001)

University of Tennessee (Business Administration)
Ohio State University (Sociology)
University of Maryland-College Park (Women's Studies)
Penn State (Education)
Ohio State University (Music Education)
DePaul University (Philosophy)
St. John's University (Clinical Psychology)
University of Pennsylvania (Political Science)

Interactions between Bordetella bronchiseptica and its Host

Luis M. Agosto, McNair Scholar, Penn State

**Myliisa R. Pilonie, PhD student in Biochemistry, Microbiology and Molecular Biology,
Penn State**

**Dr. Eric T. Harvill, Associate Professor of Microbiology and Infectious Disease,
Penn State**

Bordetella bronchiseptica is a common mammalian respiratory pathogen and is the closely related progenitor of the human pathogen *Bordetella pertussis*, the organism responsible for whooping cough. Mice are a natural host of *B. bronchiseptica*, making them an appropriate model for the study of many aspects of the bacterium's biology and its interactions with host cells. In this study the types of host cells present in the lungs early in the infection and the effects of bacterial virulence factors on phagocytic cells were determined. In addition, the wild type *B. bronchiseptica* strain RB50 was transformed with the plasmid CC2, which encodes yellow fluorescent protein, for future observation of direct bacterial-host interactions.

Introduction

Bordetella bronchiseptica, the pathogenic agent responsible for kennel cough in dogs and rhinitis in pigs, is a gram-negative coccobacillus closely related to the human pathogen *B. pertussis*, which is responsible for whooping cough. These organisms share several virulence factors, such as adenylate cyclase toxin and the attachment protein filamentous hemagglutinin. These virulence factors are transcriptionally regulated by the Bvg AS signal transduction system (Salyers 2002). This system senses environmental conditions and mediates the transition between infectious (Bvg +) and non-infectious (Bvg -) phases of the organism (Yuk 1998, 945). In the case of *B. bronchiseptica*, this system also regulates the expression of a type III secretion system (TTSS) (Yuk 1998, 945). The TTSS forms a needle-like protein structure on the surface of many pathogenic microorganisms and it is used to introduce toxins or other proteins directly into the cytoplasm of host cells. It has previously been documented that this secretion system induces caspase-1 independent necrosis of epithelial cells and macrophages in culture, suggesting it is very important for the pathogenicity of the organism (Yuk 1998, 945). Our group determined which host cells are present in the lungs early in the infection and studied what type of cell death mechanism TTSS induces on phagocytic cells. In addition, the wild type strain RB50 of *B. bronchiseptica* (Cotter 1994, 3381) was also transfected with the plasmid CC2, which encodes yellow fluorescent protein (YFP). To validate the ability of the transfected bacteria to colonize the respiratory track of its natural host, the mouse, we compared the bacterial population of YFP labeled bacteria found in the lungs to that of unlabeled RB50. Since it appears that the presence of YFP does not alter the ability of the bacteria to infect the respiratory tract of mice, we believe that this system can be used to visually examine interactions between the bacteria and the early inflammatory cells.

Materials and Methods

Host Cell Population. Three C57BL/6 mice, obtained from Jackson Laboratories (Bar Harbor, ME), were sedated with isofluorane (Abbott Laboratories) and inoculated intranasally with 5.0×10^5 colony forming units (CFU) of RB50 diluted into a volume of 50 μ L of phosphate-buffered saline (PBS). The host cells present in the lungs on day 3 post-inoculation were determined from lung homogenates. The lungs were homogenized in 10ml of DMEM plus 10% Fetal Calf Serum (FCS) through a CollectorTM screen (Bellco Glass, Inc.). 5 μ g of DNase were added to the lung homogenate to decrease the level of cell clumping. 5ml of the homogenate were added to 6ml of the Histopaque[®] 1119 gradient (Sigma) and centrifuged at 3000rpm for 30min. The visible layer of cells close to the top of the gradient was removed. 300 μ L of this cell suspension were added to the CytoSpin[®] glass slide apparatus, centrifuged for 30min at 300rpm and Giemsa stained. The different leukocyte types were counted by microscopy to a total of 300 cells per slide.

Neutrophil Isolation. Neutrophils were isolated from C57BL/6 mice blood in a 1:1 mixture of Histopaque[®] 1077 and 1119 gradients (Sigma). The mixture was centrifuged at 3000rpm for 30min. The visible layer of cells close to the top of gradient was removed. Cells were centrifuged at 13000rpm for 1min to replace the gradient with DMEM/F12 medium without phenol red plus 10% FCS.

Detection of Cell Death Mechanisms. To determine the cell death mechanism induced by RB50 on host phagocytic cells, 10^6 C57BL/6 mouse neutrophils and 10^6 cells of the RAW 264.7 mouse macrophage cell line (**Ralph 1977, 950**) were infected with heat-killed RB50, live RB50 or the type III secretion system mutant WD3 (only RAW 264.7) (**Yuk 1998, 945**) at a multiplicity of infection (MOI) of 30 for 2hr at 37°C. To determine the cell death mechanism induced, the cells were centrifuged at 5000rpm for 1min and washed once with PBS. They were then resuspended in 100 μ L of Annexin-V binding buffer (10mM HEPES pH 7.4, 140mM NaCl, 2.5mM CaCl₂, and 0.1% BSA). 0.5 μ L of 40ng/ml Annexin-V-FITC solution, a kind gift of Dr. Margaret S. Halleck, and 1 μ L of 1.0mg/ml propidium iodide (PI) solution were added to the cell suspension and incubated for 5min at room temperature. After the incubation, 500 μ L of binding buffer were added. The samples were kept on ice, protected from light and a total of 20000 cells per sample were screened by FACScanTM flow cytometry (Becton Dickinson).

Bacteria and Plasmid. The wild type strain of *B. bronchiseptica* RB50 (**Cotter 1994, 3381**) was transformed by bacterial mating with the *E. coli* strain SM10 carrying the plasmid CC2 encoding YFP (fig.1, 2), a gift from Dr. Peggy Cotter. Proper insertion of the plasmid into the bacterial chromosome was confirmed with polymerase chain reaction (PCR) using the primers CWSSHR3 and CWBpGR3, also gifts from Dr. Peggy Cotter, under standard conditions. Bacterial fluorescence was also confirmed via fluorescent microscopy. Successfully transformed bacteria were maintained on Bordet Gengou (BG) agar (Difco) with 7.5% sheep blood, 20 μ g/ml of streptomycin and 60 μ g/ml of gentamycin. Bacteria were grown in Stainer-Scholte broth with 60 μ g/ml of gentamycin prior to mouse inoculation.

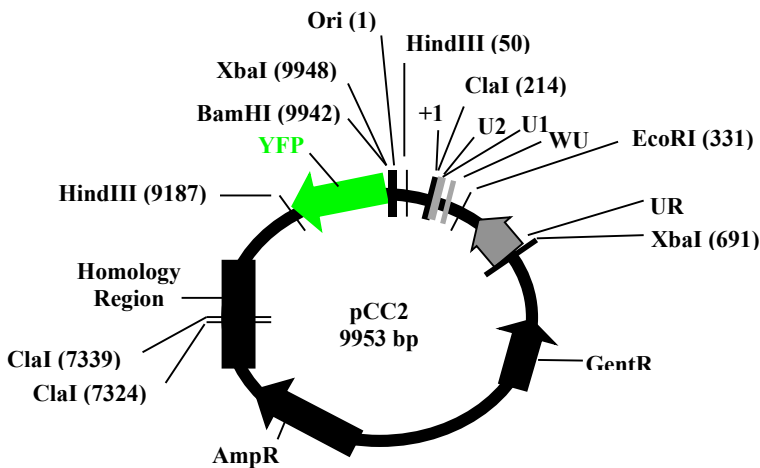


Figure 1.

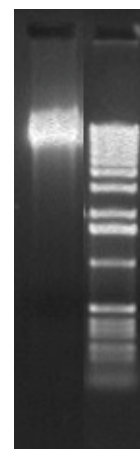


Figure 2.

Figure 1. Genetic map of the plasmid CC2 that encodes yellow fluorescent protein.

Figure 2. 1% agarose gel with purified plasmid CC2 (lane 1) and a 10 kbp standard DNA ladder. The plasmid is 9953 base pairs long.

Mouse Experiments with RB50-YFP. C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). 20 mice were sedated with isoflurane (Abbott Laboratories) and intranasally inoculated with 5.0×10^5 colony forming units (CFU) of RB50-YFP diluted into a volume of 50 μ L of phosphate-buffered saline (PBS). 4 mice per time point were sacrificed on days 1, 3, 7, 14, and 28 to determine bacterial CFU from the lungs. The organs were homogenized in 1 ml of PBS, diluted and plated on BG agar with 7.5% sheep blood and 20 μ g/ml of streptomycin for 48 hr at 37°C for CFU counts. The data obtained were compared to previously recorded data of RB50 in C57BL/6 mice (Kirimanjeswara 2003, 1719).

Results

Host Cell Population. The host immune system responds quickly to the presence of the pathogens in the respiratory track. To determine which cell types respond to the infection by day 3 post-inoculation we prepared slides with lung homogenates. At this time point, the vast majority of the cells were macrophages (65%), followed by neutrophils (20%) and lymphocytes (15%), (fig. 3).

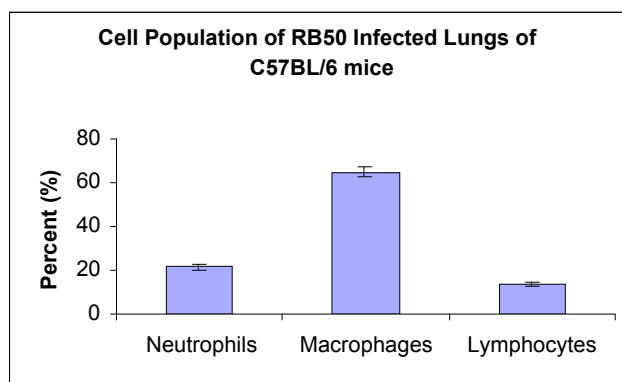


Figure 3. Host immune cells present in the lungs of RB50 infected C57BL/6 mice on day 3 post-infection. Data is expressed in average \pm standard error.

Cell death mechanism. Since previous experiments have indicated that *B. bronchiseptica* induces a vigorous cellular response during infection, the interactions of *B. bronchiseptica* with immune cell populations were examined. FITC-labeled Annexin-V was used to detect apoptosis and PI was used to detect necrosis of neutrophils and macrophages induced by RB50 infection in-vitro. First, of the RB50 infected neutrophils screened, 30% underwent apoptosis and 32% underwent necrosis. These results are significantly higher than those observed for heat-killed bacteria infected neutrophils: 14% apoptosis, 3% necrosis (fig.4). Second, in the case of the RB50 infected RAW 264.7 macrophages, 43% were observed to undergo necrosis while only 5% underwent apoptosis. The level of necrosis induced by RB50 is considerably higher than the level of apoptosis or necrosis induced by heat-killed bacteria (3% and 2% respectively) or WD3 (4% and 3% respectively) (fig.5).

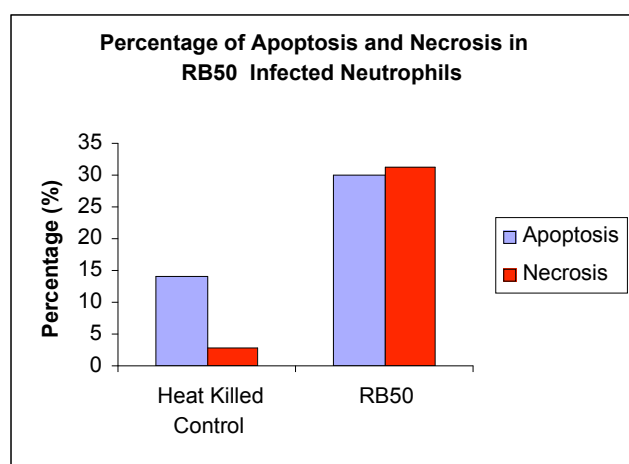


Figure 4.

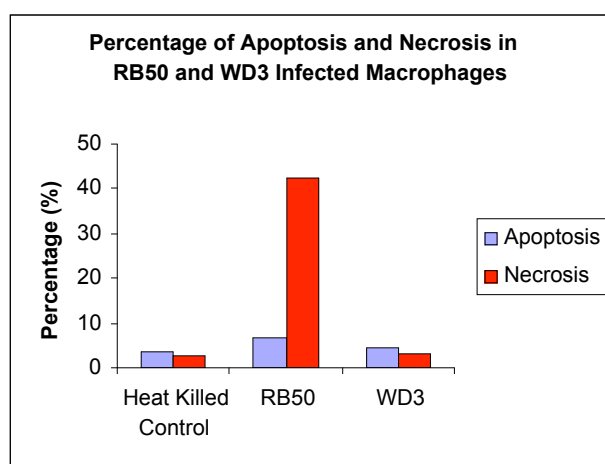


Figure 5.

Figures 4 and 5. The cell death mechanism induced by RB50 infection was analyzed by flow cytometry. Apoptosis was detected with FITC-labeled AnnexinV and necrosis was detected with propidium iodide. A total of 10^6 neutrophils isolated from C57BL/6 mice and macrophages of the RAW 264.7 mouse cell line were infected and 20000 cells were screened with flow cytometry.

pCC2 Insertion and Expression. In order to visually examine the interactions of *B. bronchiseptica* with responding host cells, the pCC2 plasmid, containing the YFP gene was transfected into wild type strain RB50 of *B. bronchiseptica*. After bacterial mating, several transformants were obtained. The transformants were subcultured on BG agar with gentamycin and streptomycin for 48hr and a colony from the subculture was selected for PCR screening to confirm proper insertion of pCC2 into the bacterial chromosome. The plasmid was inserted properly as it can be observed on figure 6. Proper expression of YFP was confirmed by microscopy (fig. 7)

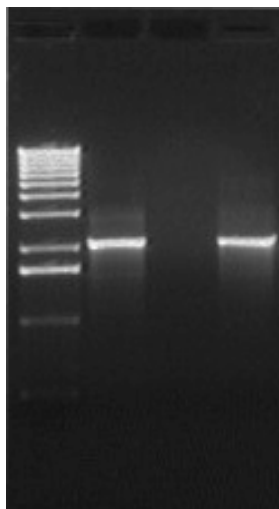


Figure 6.

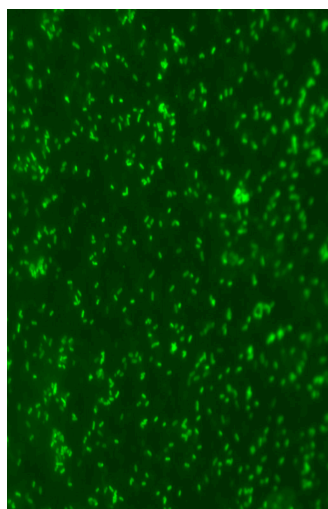


Figure 7.

Figure 6. 1% agarose gel with 10 kbp standard DNA ladder (lane 1), PCR positive control product of integrated plasmid (lane 2), negative control of integrated plasmid (lane 3), and pCC2 integrated plasmid (lane 4). PCR was carried out under standard conditions to confirm proper integration of pCC2 into the bacterial chromosome using the primers CWSSHR3 and CWBpGR3. Integrated plasmid is 2.1 kbp long.

Figure 7. Fluorescent micrograph of *B. bronchiseptica* strain RB50 expressing YFP.

Bacterial CFU. To confirm that the expression of YFP would not alter *B. bronchiseptica* colonization, bacterial numbers in the lungs of mice infected with either the wild type or YFP expressing strains of *B. bronchiseptica* were compared. After 28 days of infection, no significant difference in bacterial population between RB50 and RB50-YFP infected lungs was observed (fig.8).

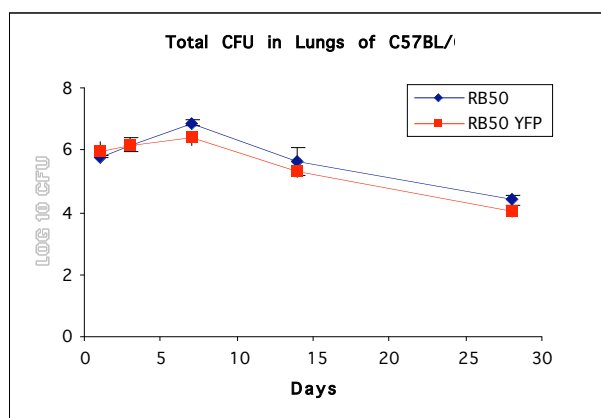


Figure 8. Infection time course of C57BL/6 mice with RB50-YFP compared to wild type RB50. Data is expressed in average \pm standard error.

Discussion and Conclusions

In this study, we determined which host cells respond to the *Bordetella* infection on day 3 post-infection from the observation of leukocytes isolated from infected lung homogenates. With this technique we found that the majority of the cells present at this stage of the infection are macrophages followed by neutrophils and lymphocytes. This technique will help us determine which cells are present throughout the course of infection.

Since macrophages and neutrophils are the main host immune cells that interact directly with the bacteria, we explored what were the effects of this interaction on these cell types. It has been reported previously that the expression of a type III secretion system by *Bordetella bronchiseptica* induces caspase-1 independent necrosis on epithelial cells and macrophages in-vitro (Stockbauer 2003, 123). With the use of Annexin-V-FITC to detect apoptosis and propidium iodide to detect necrosis through flow cytometry, we confirmed these findings in-vitro with the RAW 264.7 mouse macrophage cell line and purified neutrophils from C57BL/6 mice. We compared the cell death mechanisms induced by the wild type *B. bronchiseptica* strain RB50 and the type III secretion system mutant WD3 on RAW 264.7. We found that a significantly higher amount of necrotic cell death was induced by RB50 compared to the level of apoptosis. This level of necrotic cell death was also significantly higher than the level of necrosis induced by WD3. In the case of neutrophils, very similar levels of apoptosis and necrosis induced by RB50 were observed, however neutrophils undergo phagocytosis-mediated apoptosis (Kobayashi 2002, 6901). This could explain the relatively high level of apoptosis induced by the heat-killed bacteria (~15%) and the high level of apoptosis observed in RB50-infected neutrophils. In order to confirm this hypothesis, further experiments should be carried out. Experiments should also be done to determine what cell death mechanism WD3 induces on neutrophils.

The expression of yellow fluorescent protein by *B. bronchiseptica* is a very useful technique that will allow us to visualize many of the interactions that occur between RB50 and its host during the course of infection. This technique would also be useful to better understand other pathogens. In our study, we successfully transformed the organism with the plasmid CC2, which encodes yellow fluorescent protein (YFP). We showed, with the use of standard PCR techniques, that this plasmid integrated efficiently into the *Bordetella* chromosome. However, proper insertion of the plasmid into the bacterial chromosome does not guarantee normal colonization of the organism. To address this question we carried out a 28 days mouse infection time course and compared the total CFU of bacteria on days 1,3,7,14, and 28 with previously recorded data (Kirimanjeswara 2003, 1719). No significant difference between RB50-YFP and RB50 bacterial numbers was observed. This suggests that the integrated plasmid does not affect the ability of the organism to colonize the lower respiratory track of mice. However, in order to compare the two strains with higher accuracy, both time courses must be carried out side-by-side.

In conclusion, our group determined that most of the host cells that respond to the infection on day 3 post-inoculation are macrophages. We determined that the bacterium induced mostly necrosis on neutrophils and macrophages mediated by the type III secretion system, although further studies should be carried out to confirm these results. We also genetically

modified the wild type strain RB50 of *Bordetella bronchiseptica* with the plasmid pCC2 to induce the expression of yellow fluorescent protein. This technique would enable us to observe direct interactions between the bacteria and the host immune system during the course of infection.

Acknowledgements

I would like to thank Dr. Eric T. Harvill and the members of his laboratory for the opportunity to join his research team. Special thanks to Mylisa R. Pilione for the opportunity to collaborate with her work, Girish S. Kirimanjeswara and Paul Mann for providing useful data and help with mouse experiments, Dr. Peggy Cotter for providing the YFP plasmid pCC2 and the primers CWSSHR3 and CWBpGR3, Dr. Margaret S. Halleck for providing Annexin-V, Dr. Elaine Kunze and Susan F. Magargee for their help with flow cytometry and microscopy, and the McNair Scholars Program and all its members for giving me the opportunity to participate in the summer program.

References

1. Cotter PA, Miller J F. 1994. Bvg AS-mediated signal transduction: analysis of phase-locked regulatory mutants of *Bordetella bronchiseptica* in a rabbit model. Infection and Immunity 62: 3381-3390.
2. Kirimanjeswara GS, Mann PB, Harvill ET. 2003. Role of antibodies in immunity to *Bordetella* infections. Infection and Immunity 71(4): 1719-24
3. Kobayashi SD, Voyich JM, Buhl CL, Stahl RM, DeLeo FR. 2002. Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. Proceedings of the National Academy of Science 99(10): 6901-6
4. Ralph P, Nakoinz I. 1977. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. Journal of Immunology 119: 950-954.
5. Salyers AA, Whitt DD. 2002. Bacterial Pathogenesis: A molecular Approach, (2nd Ed.) Washington DC, ASM Press
6. Stockbauer KE, Foreman-Wykert AK, Miller JF. 2003. *Bordetella* type III secretion induces caspase 1-independent necrosis. Cell Microbiology 5(2): 123-32
7. Yuk MH, Harvill ET, Miller JF. 1998. The Bvg AS virulence control system regulates type III secretion in *Bordetella bronchiseptica*. Molecular Microbiology. 28(5): 945

studies for in-depth understanding of their biological functions. Melanin fluorescence has been extensively studied, in solution, as a potential probe for skin diseases such as cancer. For that reason, there are major interests in differentiating between cancer and healthy tissues using mainly native melanin fluorescence as a reporter. So far, however, the experimental approaches have suffered from the lack of systematic and comprehensive investigation of the molecular dynamics of melanin in its native environment with high spatial and temporal resolution.

The long term of this objective is to elucidate the excited-state molecular dynamics of melanin under different and controlled environments. Furthermore, our goal is in-depth understanding of the correlation between such ultrafast dynamics and the structural conformations of melanin in their native biological environments for optical diagnostics of skin cancer. Here, we report our preliminary studies on oxidation effects on melanin fluorescence in solution. Hydrogen peroxide is a molecule that is well known in industry and in the household. In the body, H_2O_2 plays a dual role, as both a dangerous toxin and as a valuable signaling molecule. In this report, we have used H_2O_2 for oxidizing melanin. We also used a combination of steady-state and ultrafast time-resolved fluorescence microscopy and spectroscopy techniques. The key advantage of those techniques is providing quantitative biomolecular dynamics, with both spatial and temporal resolution of biomolecules (melanin in this case) in their native biological environments. In this report, we provide preliminary results on synthetic melanin, *in vitro*, using ultrafast laser spectroscopy. These studies were carried out under different oxidation conditions to test the potential of melanin fluorescence as a reporter for different physiological conditions in living skin tissues. This characterization is critical as an early optical probe of skin cancer compared to harmless skin lesions. Our long term goal is to extend this study of melanin fluorescence to *in vivo* using the endogenous fluorophore for early skin cancer detection.

II. MATERIALS AND METHODS

II.a Chemicals: Synthetic melanin and the oxidation agent (hydrogen peroxide, H_2O_2) were purchased from Sigma-Aldrich. The non-oxidized melanin was maintained in different solvents such as dimethylsulfoxide (DMSO), phosphate buffer saline (PBS), and water. The procedures of oxidation for melanin were followed by Kayatz et al. (2001). The complete oxidation of synthetic melanin takes approximately 3 ½ - 4 hours when isolated in 30 % hydrogen peroxide.

II.b Absorption and Fluorescence Measurements: The absorption spectra of *in vitro* melanin was measured and recorded on a spectrophotometer (Model DU800 from Beckmann and Coulter) over a 300-800nm range as a function of oxidation and solvent type. Fluorescence spectra were measured with a fluorimeter (Spex Fluorolog F212) using an excitation wavelength of 470 nm with a 10-nm bandpass, and 1.5 nm resolution. Steady state anisotropy measurements were also recorded using the emission wavelength at 540 nm.

II.c Time-trace of Melanin Oxidation using Steady State Spectroscopy: In order to investigate the oxidation effect on melanin fluorescence, we have measured time traces of the absorption and emission spectra as a function of time after melanin oxidation by incubation in 30% aqueous solution of hydrogen peroxide. Oxidation time-traces were followed approximately 200 minutes for both fluorescence and absorption spectroscopy. The first time point for both spectra was measured approximately 15 minutes after melanin had been in the hydrogen peroxide solution (~15 min to dissolve).

II.d Time resolved Anisotropy and Lifetime Decay Measurements: Time Correlated Single Photon Counting module (SPCM830; Becker and Hickl) was used for time resolved fluorescence and anisotropy measurements. The sample was staged on an inverted microscope (Olympus IX81) for recording the fluorescence decays at parallel, perpendicular, and magic-angle polarizations with respect to the excitation laser.

III. RESULTS AND DISCUSSION

III.a Steady State Time-Traces of In Vitro Melanin Oxidation: Synthetic melanin exhibits low solubility in organic solvents (e.g., DMSO and PBS) with an absorption spectrum similar to that shown in Figure 2.a. In contrast, the oxidized melanin was dissolved completely within ~15 minutes in hydrogen peroxide with similar absorption spectra. While oxidization does not affect the absorption spectrum of melanin, it does reduce the optical density compared with non-oxidized melanin.

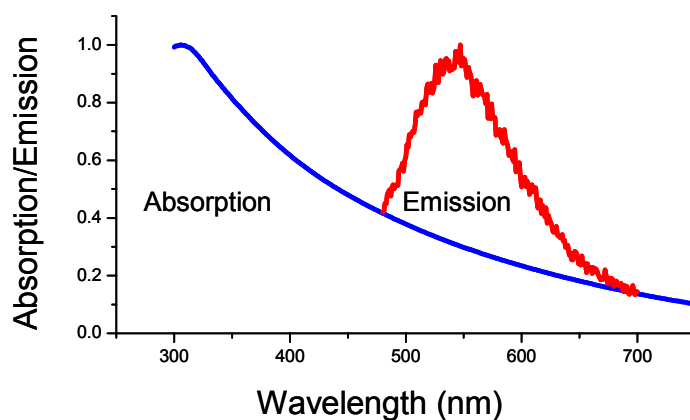


Figure 2.a: Absorption and emission spectra of melanin in DMSO.

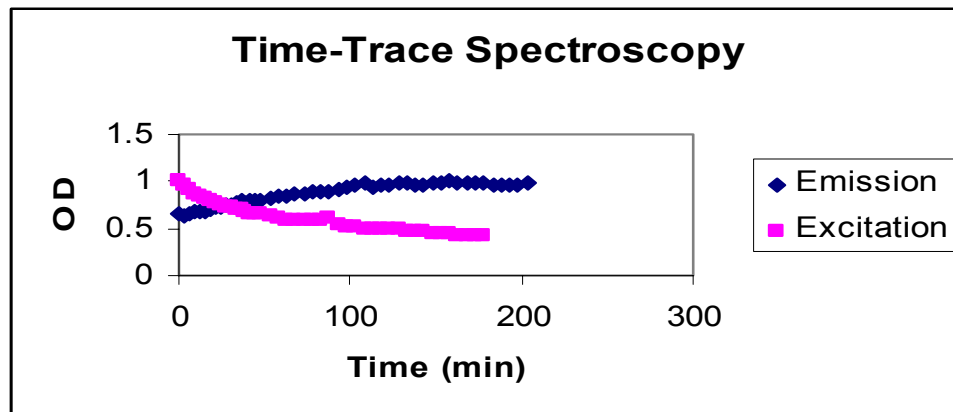


Figure 2.b: Time traces reveal oxidation effects on the absorption (squares) and emission (diamonds) of melanin.

Furthermore, the fluorescence emission (~ 540 nm) is also enhanced upon oxidation (see Figure 2.b for absorption and emission as a function of the oxidation time-traces). This is consistent with the argument that oxidation of *in vitro* melanin degraded the melanin polymer, resulting in an efficiently fluorescent molecule (Kayatz et al, 2001). This means that polymeric melanin causes fluorescence quenching of the non-oxidized fluorophores. To test this argument, we measured the excited-state fluorescence lifetime, which is independent of the fluorophores concentration and also directly proportional to the fluorescence quantum yield.

III.b Oxidation Affects on the Excited State Dynamics of Melanin: The fluorescence of melanin decays (under 495 nm excitation) as a triple exponential, independent of the solvent (HP, DMSO, or PBS) as shown in Figure 3 and Table 1. However, the average excited state lifetime of oxidized melanin is distinctively different from the non-oxidized melanin by at least a factor of 2. The incubation time of melanin was long enough to guarantee that oxidation reaction was complete. Under the same excitation conditions, the fluorescence was negligible in water.

The multiexponential fluorescence decays (see Table 1) suggest the presence of multiple species or electronic-state transitions of melanin, independent of the solvent or oxidation (Figure 3). However, the slower fluorescence decay of the oxidized melanin suggests an enhancement of the fluorescence quantum yield, which is consistent with our steady state spectroscopy measurements. By comparison, observed the faster decays observed in DMSO and PBS (non-oxidizing solvents) our argument that the polymerization of melanin causes fluorescence quenching, under non-oxidizing conditions, which compete with fluorescence.

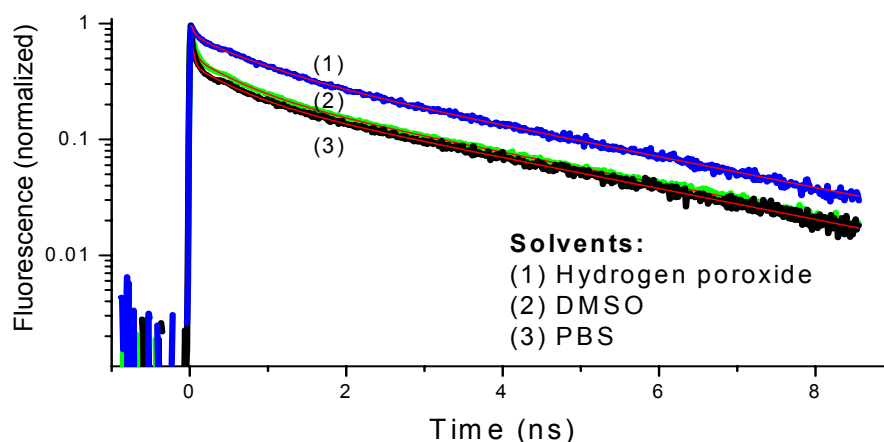


Figure 3. Excitation dynamics with no effects of polarization. A measure of the normalized fluorescence lifetime decay of the oxidized and non oxidized conditions. For the fitting parameters, please see Table 1.

To further test our hypothesis that there is a correlation between the observed fluorescence enhancement and the conformational structure of melanin, we measured time-resolved fluorescence anisotropy of melanin as a function of solvent type (oxidizing versus non-oxidizing).

Table 1. Fitting Parameters of the fluorescence lifetime decays of melanin in hydrogen peroxide (HP), DMSO, and PBS solvents.

Fitting Parameters	HP	DMSO	PBS
Time constant (τ_1)	51 ps	38 ps	29 ps
Amplitude (a_1)	0.21	0.48	0.52
Time constant (τ_2)	794 ps	616 ps	606 ps
Amplitude (a_2)	0.34	0.25	0.20
Time constant (τ_3)	3.26 ns	3.24 ns	3.26 ns
Amplitude (a_3)	0.45	0.26	0.24
Average Lifetime $\langle\tau_{fl}\rangle$	1.75 ns	0.84 ns	0.92 ns

III.c Oxidation Effects on the Rotational Diffusion of Melanin: To further understand the mechanism that underlies both solvent and oxidation effects on the absorption and emission properties, we have measured time-resolved fluorescence $I(t)$ and anisotropy $r(t)$ using time-correlated single photon counting technique. We used the measured parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ fluorescence polarizations to calculate the anisotropy decay and, therefore, the rotational time of melanin, which is a sensitive

probe to both the environmental viscosity (η) and the hydrodynamic volume (V) of the fluorophores:

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} = r_0 \exp(-t/\phi), \quad \text{and} \quad \phi = \eta V / RT$$

where the denominator in the $r(t)$ -function equal to the magic angle fluorescence decay and r_0 is the initial anisotropy. Also R and T are the gas constant and temperature (in Kelvin) of the solvent.

Figure 4 exhibit the oxidation effect on the rotational diffusion of melanin (see the figure caption for the fitting parameters). The anisotropy decays of oxidized melanin demonstrate clearly the degradation of melanin polymerization, which is present in non-oxidized melanin when dissolved in DMSO or PBS. Oxidation of Melanin affects its structural conformation. The anisotropy of melanin decays as a biexponential but the decay parameters are dependent on oxidation. Anisotropy of melanin in DMSO (*Black curve*) decays with two rotational time constants: fast component with $\phi_1 = 640$ ps ($a_1 = 0.25$) and slow component $\phi_2 = 2.03$ ns ($a_2 = 0.06$). Anisotropy of melanin in hydrogen peroxide (*Blue curve*) decays also as a biexponential, but with two different rotational time constants: $\phi_1 = 95$ ps ($a_1 = 0.12$) and slow component $\phi_2 = 443$ ps ($a_2 = 0.17$). While the initial anisotropy (r_0) is about the same, the rotational time of oxidized melanin is ~ 1.6 time faster than the non-oxidized fluorophore. This small ratio of rotational time of polymerized-to-non-polymerized melanin might suggest a smaller polymerization extension.

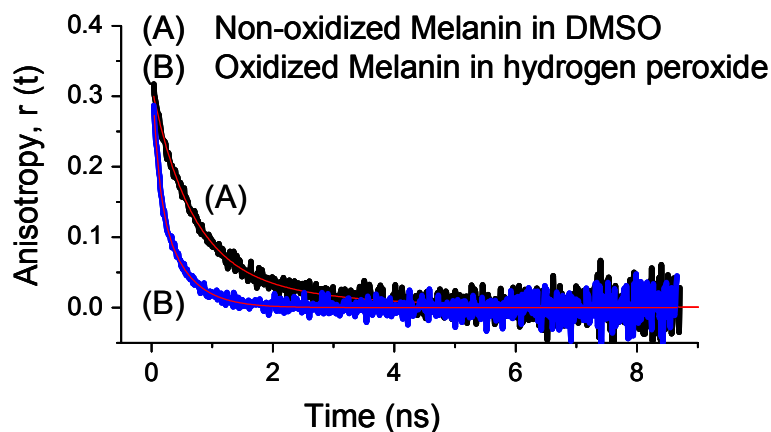


Figure 4. Oxidation of Melanin affects its structural conformation. The anisotropy of melanin decays as a biexponential but the decay parameters are dependent on oxidation (Curves A and B are for non-oxidized and oxidized melanin, respectively). See Table 2 for the fitting parameters.

Table 2. Fitting Parameters of the fluorescence anisotropy decays of melanin in hydrogen peroxide (HP) and DMSO.

Fitting Parameters	DMSO	HP
Rotational Time (τ_1)	640 ps	95 ps
Amplitude (a_1)	0.25	0.12
Rotational Time (τ_2)	2.03 ns	443 ps
Amplitude (a_2)	0.06	0.17
Initial Anisotropy (r_0)	0.31	0.29

These results confirm our assertion that the enhancement of melanin fluorescence properties upon oxidation can be understood in terms of structural conformation changes.

CONCLUSION

We investigated the oxidation effects of melanin fluorescence properties using steady-state and time-resolved fluorescence techniques. The enhanced fluorescence of oxidized melanin is attributed to the degradation of melanin polymerization. The longer excited state lifetime of oxidized melanin suggest that melanin stacking in a polymer causes fluorescence quenching. This argument is supported by the time-resolved fluorescence anisotropy, which suggest a much slower rotational time of melanin under non-oxidizing conditions (i.e., polymerized melanin). However, the multiexponential decays also suggest multiple structural conformers of melanin under oxidizing or non-oxidizing conditions. Our ultimate goal is to use the preliminary results and understanding of the sensitivity of melanin to the local environment to distinguish between cancerous and non-cancerous cases of skin.

ACKNOWLEDGEMENTS

I would like to express sincere thankfulness to the McNair Scholars Program Staff for all of their support and help. I would also like to extend thanks and appreciation to Dr. Ahmed Heikal, my FRA in the Bioengineering Department at The Pennsylvania State University, Laboratory for Functional Imaging of Biomolecules. Last but not least, the help and support of the graduate students in the lab, Ronn Walvick and Deepti Mudaliar, as well as the other 2004 summer research internship students in Dr. Heikal's group.

REFERENCES

1. Klaus Hoffmann, Markus Stücker, Peter Altmeyer, Klaus Teuchner, and Dieter Leupold. Selective Femtosecond Pulse-Excitation of Melanin Fluorescence in Tissue. *J. Investigative Dermatology* 116 (4), 629-630 (2001).
2. Klaus Teuchner, Jurgen Ehlert, Wolfgang Freyer, Dieter Leupold, Peter Altmeyer, Markus Stucker, and Klaus Hoffmann. *J. of Fluorescence* 10:275-281 (2000).
3. Peter Kayatz, Gabriele Thumann, Thomas T. Luther, Jens F. Jordan, Karl Ulrich Bartz-Schmidt, Peter J. Esser and Ulrich Schraermeyer. Oxidation Causes Melanin Fluorescence. *Investigative Ophthalmology & Visual Science* Vol (42) No. 1, 241-246 (2001).

Assessing Parental Beliefs in Early Childhood

Trinity Crosby, McNair Scholar, Penn State

Dr. Douglas M. Teti, Professor, Human Development and Family Studies
Penn State

Abstract

Assessing parental beliefs in early childhood is significant because of its ability to properly understand parenting as an adaptational process along with child development. Sigel and McGillicuddy-De Lisi (2002) stated, “What parents believe about parenting is related to their own parenting and to virtually every aspect of children’s developmental accomplishments.” The purpose of this study is to identify maternal beliefs about competent parenting in early childhood. The present study will look at maternal beliefs relating to parenting with a six-month old as well as parenting as an adaptational process.

INTRODUCTION

Every mother has her own belief on what competent parenting consists of. Assessing mother’s beliefs on competent parenting is part of measuring parental competence. What mothers believe parenting to be is directly related to their competence, and how they parent their child. Teti & Huang (2004) suggest, in order for a parent to be competent they must be able to change their style of parenting as it applies to their child’s development. According to Mary Ainsworth (Ainsworth et al. 1978), competent parenting during infancy consists primarily of love and nurturance. This parent is sensitive to their child’s needs and answers to them in a timely and effective manner. Teti & Huang (2004) also states that parenting competence should change qualitatively in transition from infancy to preschool years. These researchers felt that parents who were competent during infancy would remain so throughout their child’s development. The purpose of this study is to identify maternal beliefs about competent parenting in early childhood.

In most cases, the first few years of a child’s life are spent at home with their parents or legal guardians. Since a child is constantly developing and changing, it is important that the parent as the primary caregiver is also changing in their parental styles and making the proper adjustment in order to help the child develop most effectively.

The Problem

Parenting is a dynamic adaptational process, which develop in accordance to the developing needs of the child. Not all parents may understand how imperative it is to adjust their parental behavior to the development of the child. Some parents may feel that parenting behavior should be consistent and it is not necessary to change as the child develops. Other parents may understand the importance of adjustment; however, they do not adjust their parenting in a manner that promotes healthy parent-child relationships

and child development. As a result, the child is being negatively affected in their socio-emotional and intellectual competence.

Significance of the Study

In today's society, the phenomenon "Children are the future" is widely known across the nation. The children of today are the leaders of tomorrow. They will be the future doctor, lawyer, and even president to whom we depend. Therefore, it is the parent's job to shape and mold their child in a way that will promote positive development so that the child will become a positive influence and role model as they grow older. The way in which a child grows and develops is dictated and influenced in part by the beliefs of the parent, which in turn is expected to influence actual parental behavior. Studying parental beliefs in early childhood is part of the foundation to understanding parental behavior and children's social and cognitive development.

Research Questions

This study will answer the following questions:

1. What are mother's beliefs on good parenting as it applies to their child?
2. Do mothers feel it is imperative to adapt their parenting style and behavior as their children grow and develop? If so, how?
3. How knowledgeable are mothers as it applies to parenting their child?

Conceptual Framework

This study relies on the work of McGillicuddy-De Lisi and Sigel, who have studied parental beliefs and parental beliefs system for quite some time. Their research assumes that every parental belief leads to the parental behavior, which then yields a particular child outcome (McGillicuddy-De Lisi & Sigel 1995). Additionally, their research has defined eight beliefs, which applies to all parents when it comes to their particular parenting style. The eight beliefs are described below.

1. "Beliefs are knowledge based and are constructions of experience. The cognitive processes involved are assimilation and accommodation (k)" (McGillicuddy –De Lisi & Sigel, 2002, p.499).
2. "Beliefs are organized into categories of knowledge domains of which are bounded. The internal coherence of the domains may vary and the boundaries may vary in permeability (D)" (Mc Gillicuddy-De Lisi & Sigel, 2002 p.499)
3. Beliefs may be held as absolutes or as probabilities (A/P)" (Mc Gillicuddy –De Lisi & Sigel, 2002 p. 499).
4. "Beliefs serve comparable functions for everyone irrespective of culture, although the content and experiential bases from which beliefs are constructed within a culture milieu are different (C)" (McGillicuddy –De Lisi & Sigel, 2002. p.499)
5. "Beliefs are influenced by affect to varying degrees of intensity and quality of influence (A)" (McGillicuddy –De Lisi & Sigel, 2002, p.499).
6. "Beliefs are expressed in behaviors that may have one or more goals (G)" (McGillicuddy –De Lisi & Sigel, 2002, p. 499).
7. "Beliefs vary in the degree to which they are valued (i.e. deemed important) (V)" (McGillicuddy –De Lisi & Sigel, 2002, p. 499).

8. “There is a subset of beliefs derived from core beliefs as to how and under what conditions to instantiate actions to express the core beliefs. These are the praxis beliefs about how and in what form beliefs should be enacted. One important category of praxis beliefs is beliefs in one’s ability to effectively generate and implement a parenting strategy, as well as other aspects of parenting self-efficacy (SE)” (McGillicuddy –De Lisi & Sigel, 2002, p. 499)

REVIEW OF RELATED LITERATURE

The purpose of this study is to identify maternal beliefs about competent parenting in early childhood. In addition, the present study will identify the mother’s belief on parenting as an adaptational process, and whether or not they feel that this is something that they will practice as a parent. One of the elements to assessing parental beliefs is to identify where these beliefs may come from and how they are influenced. In addition, the assessment of parental beliefs can lead to the prediction of what parenting style is going to be used by certain parents.

Sources of Parenting Beliefs

Parental beliefs can be influenced and determined by a variety of different factors. Parental beliefs may be determined by one’s environment, age, ethnicity, and even his/her experience of just being a parent (Scott & Hill 2001). According to Meriam and Webster, environment is defined as “the circumstances, objects or conditions by which one is surrounded (Meriam-Webster Online 2004). Some parents may find their own particular beliefs to be altered by advice from their relatives; such as a parent or aunt and uncle. Other parents may define their beliefs according to what they see other parents are doing (Smetana 1994). In a previous study, Cohen (1981) found that some parents, who are inexperienced, actually tend to look to other parents’ beliefs and what they practice in order to help identify their own beliefs. It is also evident that parental beliefs may be determined by one’s age. In today’s society, there are a number of mothers who are having children at a young age and who are therefore limited when it comes to parenting. These particular mothers may exhibit different beliefs about parenting opposed to older mothers due, to the fact that they are much younger. Fry (1985) conducted a study on maternal beliefs with younger mothers and compared them to older mothers. In conclusion his study found that younger teenage mothers compared to older mothers actually showed less knowledge of child development. Additionally, the younger mothers did not believe it was necessary to show a lot of verbal interaction with the child.

Ethnicity and culture seem to play a huge role in parental beliefs (Cote & Bornstein 2000). For example, Latino cultures are believed to place high value on interpersonal relationships but retain significant independent qualities (Parke & Buriel 1998). Although Asian cultures value independence they place more value on interdependence with others. This culture strongly believes in the family unit and depending on one another as a family. Therefore, when it comes to raising children, they will instill beliefs of interdependence with the family (Markus & Kityama 1991). Uba (1994) compared maternal beliefs between Japanese-American and European-American mothers in early childhood. Results have shown that Japanese-American

mothers value social competence, and, therefore engage in more social interactions with their infants as opposed to European American mothers. When studying maternal beliefs, it is very important to take into consideration all of the determinant facts that will cause individual differences. Because of the cultural difference in parental beliefs many children are raised differently and learn to appreciate different values.

Parenting Styles

Parental styles differ for each individual and are congruent to their own circumstances and parental beliefs. There are four different styles of parenting, which were first defined by Diana Baumrind: authoritative parenting, authoritarian parenting, permissive parenting, rejecting-neglecting parenting (Smetana 1994). In her research Baumrind believed that these four different styles of parenting all followed under two different dimensions. The first dimension was demandingness, which is defined, by how demanding a parent is with their child as applied to child's behavior or actions. The second dimension was responsiveness, which is defined by how responsive the parent is when making rules (Smetana 1994). For example, if a parent tells his/her child to stop doing a particular activity, this demonstrates their level of demand. Some parents may go a little bit further once they give a child a demand and actually respond with a reason as to why he/she should not do a certain thing. The four different styles that Baumrind defined are based upon these two different dimensions.

The first style of parenting defined by Baumrind is the authoritative parenting style. This parent exhibits consistent firm regulations and control, however, they give clear explanation to their child for their standards. Authoritative parents are very loving with their children and are supportive of their child. In addition, these parents are also strong believers of autonomy for their child (Baumrind 1998). Secondly there is the authoritarian parenting style. In this style of parenting parents are very demanding of their child and are not strong believers of giving their child a response and providing them explanations. They discourage give-and-take feedback with their child. A parent with this particular style believes that it is "his/her way or no way. They tell their child what they should do and do not expect them to give any feedback. Thirdly, there is the permissive parent. This parent seems to show very little demand when it comes to following rules, and they pretty much allow their children to do what they want. Finally, the rejecting-neglecting parent is one who does not monitor their child's behavior and tends to be much disengaged with their child. They do not set any limits for what their child does. Also, they are not at all responsive, so they do not provide any type of warmth to their child (Baumrind 1988). Judith G. Smetana (1994) believes that the style of parenting will not change too dramatically over time because parenting styles are defined by parent's values, attitudes, and beliefs, and should be consistently stable over a long period of time. Teti & Huang 2004 also believed this to be true as well in the sense of competent parenting. They have stated in their research that parents who are competent in the

early years will remain so in the later years. They also feel that these parents will be very effective in making the parenting transition from infancy to preschool years.

Clinical child psychologist, Anne O'Conner (2004) states that each style of parenting has a different effect on the child and that one style of parenting is recommended as the best of the four different types. The style of parenting that produces the most positive result is the authoritative style. Children, whose parents employ this approach, have been found to be happy, self-reliant and able to cope with stress. They also tend to be popular with their peers and have good social skills and confidence. They tend to set their own standards and are achievement oriented. However, children of authoritarian parents tend to be obedient, orderly, and not aggressive. They lack the self-esteem and self-control of their peers brought up by authoritarian parents. These children do not get praised often, and as they grow older, they tend to be motivated to do things for reward or punishment, rather than for reasons of right or wrong. In contrast to other types of parenting, children raised by permissive parents do not cope with stress very well and get angry if they don't get their own way. Also, they can be aggressive and domineering with their peers and do not tend to be achievement oriented (O'Conner 2004).

METHODOLOGY

Population Sample and Recruitment

In order to assess maternal beliefs about parenting this particular study looked at mothers living in State College, Pennsylvania, and the surrounding areas. First, names and addresses of the mothers were obtained from the birth announcements in the Centre Daily Times. After receiving a list of names, an interest letter was sent out to all of the mothers, which stated the purpose of the study and who the mothers would need to contact if they were interested. Within a week of sending each mother a letter, research assistants followed up with phone calls to ascertain if the mothers received the letters and if they would agree to participate. Mothers who chose to participate in the study had to be at least 18 years of age. It was also required that their child was the first-born and was six-months old. The sample included a total of nine Caucasian mothers. All participating mothers were given a \$60.00 stipend to compensate for their time.

Instrumentation

Each mother was given a demographic sheet to be filled out prior to the parenting interview. The demographic sheet consisted of different questions that asked about the mother's marital status, age, race, employment status, highest level of education completed, estimated yearly family income, how many children the mother had, and if the child currently lived with her at the present time.

In addition to the demographic sheet, each mother answered a total of nine open-ended questions in interview form, and was asked to answer each of the questions to the best of their ability. The questions related to their idea about good parenting and

what a parent should be doing when it came to feeding and playing with their baby. This paper will only focus on two of the questions that the mother's were asked. First, "What do you think good parenting is to a six-month old like your infant is?" Second, "Right now your baby is only six-months old, but it won't be long before he/she will be a toddler, and soon after that a preschooler. Do you think your parenting will need to change, as your child gets older? If so, how?" In addition, this paper will also look at some of the beliefs and ideas that different mothers had about parenting that may be significant in helping to determine their parenting style.

RESULTS

There was a good deal of variation in mother's beliefs on parenting. First, when mothers were asked what good parenting would be for their child, some mothers seemed to emphasize one particular issue as opposed other mothers. Mothers one, two, four, five, and seven remarked that good parenting to them is showing their child a lot of love and care. In addition, it was very important to interact with their child and develop a bond between them. These mothers felt that at the age of six-months it is important for their child to develop a sense of trust with them. Mothers three and ten remarked that their idea of good parenting is exposing their child to new and different things and allowing their child to meet new people. These mothers felt that it was very important for their child to start to develop a sense of socialization. Mother six remarked that her idea of good parenting was just taking care of her baby's basic needs. Finally, Mother eight remarked that her idea of good parenting is trying to encourage her son's independence. This mother is very significant because she is the only mother who is single and not receiving any type of help in raising her child. Therefore her belief's about parenting is different from the other mothers specifically because of her circumstance of being a single mom. Also, mother eight remarked that she loves to spend time with her son and she thinks it is important, however, because she is a single mother, she tries to encourage him to do things on his own.

When asked if parenting should change when their child got older and how; mothers three, seven, and ten said that once their child got older they would practice more discipline with them. Somewhat similar, mothers two and eight said that as their child got older they would be more consistent and structured with them. These mothers felt that as a child reaches his/her toddler years they will try to test their limits and see what they can and cannot do. As a result, mothers two and eight said that it is very important to set rules and to be consistent with their child once they get older. Also, they felt that it was important to be structured and have their child on a schedule when it came to certain things, such as bedtime. Mothers four and five believed that their parenting would change because they would be considered more of a role model for their child. They also felt that it was very important to show the same amount of love, however, just try to be an example for their child. Mother one remarked that as her child gets older she will be learning and communicating differently. Therefore, this mother felt that it would be important for her to change her parenting in a way that seemed to fit with her child. Finally, mother six had a very interesting and honest response. She remarked that her parenting would have to

change when her child got older, but she had no idea how it should change because she had no type of experience with children and did not know what to expect.

The participants were also asked what a good parent should be doing when playing with an infant. Mother five believes in engaging in more one on one activities with her baby instead of relying on toys. Although, she would have toys for her child to play with, when she played with them him, she said she would do different things with him like peek-a-boo, sing songs, and other games that allowed for her and her son to develop a physical bond. On the other hand, Mother eight remarked that it was important for her to play with her son and spend time with him however, because she is a single mom, she does try to encourage him to play by himself. Also, there may be times when she may have to clean up or do some paperwork and cannot entertain her son. Therefore, this mother remarked, it is okay for her to put on baby Einstein videos to occupy her son until she is done doing what she is doing. This was a very interesting response because it does reiterate how one's own personal situation can dictate their parenting beliefs and even their behavior.

According to Baumrind's work none of the mothers that participated in the study would seem to be rejecting-neglecting parents. One of the mothers that participated could definitely be defined as one who would take on the authoritative parent due to her response on what she would do if her child made a mess. Mother eight was the only mother that said if her child makes a mess such as writing on the wall she would tell her child to clean it up but she would give her a reason. She would explain to her child that they want their house to look nice because they live there and they should take good care of it. This response is clearly one of an authoritative parent.

Limitations

As stated earlier, previous researchers have found parental beliefs to change according to one's culture, ethnicity, age, educational background, marital status, as well as their experience in being a parent. As a result of the previous findings on parental beliefs, this study has a few limitations. First, the sample size of this study consisted of nine Caucasian mothers living in State College, Pennsylvania and surrounding areas. Because of this, the results obtained from this study may or may not be applicable to mothers who are of a different race and who live in a different type of environment such as a city. Judith G. Smetana (1994) suggests that parenting styles and behaviors are partly influenced by one's environment. Second, all of the participants were over the age of 25 and had some type of college education. According to Fry (1985), the age of the mothers along with the amount of education that they possess may influence their knowledge in child development as well as their maturity level when it comes to parenting. All participants in this study were married and living with their partner, except for one mother. Some researchers have found that spousal support is very beneficial when it comes to parenting, because each spouse can share the role of what it takes to be a "competent parent", unlike single parent homes where the responsibility of caretaking is focused only on the mother or father of the child. Additional research of this particular study would need to be done in a more diverse population setting in order for the results to be generalized to all populations. This study was limited because it only looked at maternal beliefs. Although every mother had their own ideas and beliefs about parenting, it does not

mean that they would or would not necessarily practice something different. For example, in relation to ideas about good parenting, some mothers mentioned love and interaction while, other mothers just mentioned meeting new people. It would not be accurate to assume that the mothers who did not mention love felt that it was not important. These mothers may have just felt that showing love was automatic and there was no need to mention it. As a result of this, there would need to be additional research done that looks at the emotional aspect of parenting. In looking at the mother-child interaction, one can actually assess maternal behavior and emotional availability of the mother and the child and then see if it correlates with the mother's belief on parenting.

FUTURE DIRECTIONS

In the near future, the present study will expand to a total of 45 mothers, inclusive of the nine mothers that have already participated, all living in State College, Pennsylvania and the surrounding areas. The final study will include 15 mothers with a six-month old baby, 15 mothers with an 18-month old baby, and 15 mothers with a 36-month old baby. All of the participating mothers will be expected to fill out a demographic form to help researchers understand their backgrounds. The researchers will conduct a total of three visits at the mother's home. The mothers will be divided up into two different groups. For group one, the first and second visit will be 10-14 days from each other, and the third visit will be 3-5 days after visit two. For the second group, the first and second visit will be 3-5 days apart, and the third visit will be 10-14 days after visit two. During these visits, the researchers will record 25-30 minutes of the parent-child interaction during feeding and playtime. In addition to the feeding and playtime, there will also be a 25-30 minute recording of clean-up time for those mothers with an 18-month or 36-month old baby. At the end of the third visit, each of the mothers will be given a questionnaire interview that asks about their belief on parenting. The researchers will be looking at the different beliefs that mothers have on parenting and compare it to their actual interaction to assess the emotional aspect of parenting. The purpose of this expansion will be to gain an understanding of parental beliefs and how they correlate with a given behavior. It is important to assess emotional availability of the parent-child interaction in addition to assessing parental beliefs in order to identify parental competence. As stated before, some researchers believe that parental belief determines the parental behavior (McGillicuddy –De Lisi & Sigel 1995). However, it may be true that belief and behavior is actually a bidirectional entity that is, beliefs influence behavior in some instances but in other instances any given behavior will alter a particular belief.

REFERENCES

- Teti, D.M., Huang, K.Y. (2004). Developmental Perspectives on Competent Parenting
- Cote, L.R., Bornstein, M.H. (2000). Social and Didactic parenting behaviors and beliefs among Japanese American and South American mothers of infants. *Infancy*, 1 (3), 363-374.
- Markus, H.R., Kitayama, S. (1991). Culture and the self: Implications for cognition, emotion, and motivation. *Psychological Review*, 98, 224-253.
- Parke, R.D., Buriel, R. (1998). Socialization in the family: Ethnic and ecological perspectives. In W. Damon (Series Ed) & N. Eisenberg (Vol. Ed). *Handbook of Child Psychology: Vol 3. Social, Emotional, and Personality development* (5th ed. 463-552). New York: Wiley.
- Fry, P.S. (1985). Relations between teenager's age, knowledge, expectations and maternal behavior. *British Journal of Developmental Psychology*, 3, 47-55.
- Scott, D.A., Hill, J. (2001). Stability and change in parenting beliefs in first-time mothers from pre-to postnatal period. *Journal of Reproductive and Infant Psychology*, 19 (2).
- Baumrind, D. (1998). "Rearing Competent Children". In W. Damon (ed.) *Child Development Today and Tomorrow*. San Francisco: Jossey-Bass Publishers.
- Smetana, J.G. (1994). *Beliefs about Parenting: Origins and Developmental Implications*. San Francisco, Ca: Jossey-Bass Publishers.
- McGillicuddy-De Lisi, A.V., Sigel, I.E. (2002). Parenting Beliefs are Cognitions: The Dynamic Belief Systems Model. In M.H. Bornstein (Ed). *Handbook of Parenting*. Vol 3. Mahway, New Jersey: Lawrence Erlbaum Associates.

A Biomechanical Evaluation of Standing in High- Heeled Shoes

Paula D. Henderson, McNair Scholar, Penn State

**Dr. Stephen J. Piazza
Departments of Kinesiology, Mechanical Engineering, Bioengineering, and
Orthopedics and Rehabilitation
Penn State**

ABSTRACT

The purpose of this study was to determine the action of the ground reaction force upon the heels of women standing in high-heeled shoes. The study involved a non-invasive determination of the location of the subtalar joint axis, the joint about which the foot bends in and out. It was determined where the ground reaction force acts relative to the subtalar joint axis and whether muscle activity while standing depends on this result. Five healthy female volunteers will stand while wearing 2.5” heels, 1” heels, and barefoot while ground reaction forces and electrical muscle activity were recorded.

INTRODUCTION

Historical Perspective

Stilettos. Boots. Pumps. “Open toed.” Around the age of twelve, girls all over embarked on their first pair of high-heels. Historically, the first form of high heels as we know started during the 14th century. Gentry and noble men started placing wooden slips, called platens, to the bottom of their footwear to protect them from getting dirty. (Linder and Saltzman, 1998) However, in later days they have become a characteristic of femininity and an accepted custom for women in our society. Through the evolution of time, several features have made high heels distinguishable in comparison to other shoes worn by women. On the average, shoes have a heel elevation of approximately 1 to 2 cm; however, high-heeled shoes can have a heel height greater than 10 cm high. High-heeled shoes also pose a narrow toe box, a rigid heel cap that often protrudes anteriorly (Stephens, 1992) and excessive plantar curvature in the forefoot (Schwartz and Heath, 1959). Whether it is to gain a height advantage, look professional, or stay with the trend of fashion, it is not entirely uncommon for a woman to own a hundred pairs of these shoes at one time. Wearing such footwear can often have deleterious and irreversible biomechanical effects. (Linder and Saltzman, 1998) “For 250 years medical scientists

have propagandized about the health hazard of high heeled shoes, ...”. (Linder and Saltzman, 1998)

Despite the uncomfortable feelings that some women experience while wearing high heeled shoes, heels are getting higher, inclines are steeper, and toe boxes are more pointed. (Lee et al., 2001) To achieve “toe cleavage” (toes that are perfectly aligned in pointed toed shoes), as it is known to the fashion-conscious world, more than half the members of the America Orthopedic Foot & Ankle Society are responding to women risking permanent disability of cosmetic foot surgery, such as shorten toes, at a cost of \$2,300 per toe and collagen injection into the balls of the feet. The collagen serves to restore lost padding caused by frequent high heel usage and costs approximately \$500 per injection. (Harris, 2003) While ample research is done on the biomechanics of high-heeled shoes, research has yet to look at the anatomical differences and biomechanics principles of the subtalar joint axis in relation to its’ effect of standing in high heel shoes. The study will seek to answer the following questions:

1. How does the ground reaction force act upon the heels of women who wear heel-heeled shoes?
2. How does this action vary across subjects in accordance to the location of their subtalar joint axis?
3. How does muscle activity patterns correspond to the action of the ground reaction force?

Significance of the study

The data collected in this project is of relevance because the subjects- women are a large segment of the world’s population. A census brief entitled “Women in the United States: A Profile”, projected women to outnumber men by 10 million by the year 2005. (Spraggins, 2005) A 1986 Gallup Organization survey determined that 59% of the women surveyed associated wearing high heel shoes for at least one hour to more than eight hours a day. (The Gallup Organization Inc., 1986) This research can also help assist to educate women as to the effects of standing in high-heeled shoes and may positively influence the design of high-heeled shoes, which could lead towards a more comfortable and anatomically correct shoe. In addition, the study may clear up assumptions that all women should avoid wearing high-heeled shoes and formulate a link to the anatomical variations of women and effects of high-heeled shoes.

RELATED LITERATURE

Literature was reviewed in the following topic areas: subtalar joint axis, ground reaction forces, and muscular activity.

Subtalar Joint Axis

The subtalar joint (figure 1) is a composite joint formed by three separate plane articulations located superiorly to the talus and inferiorly to the calcaneus. Together, the three surfaces provide a triplanar movement around the single joint axis and one of the functional joints of the foot and ankle.

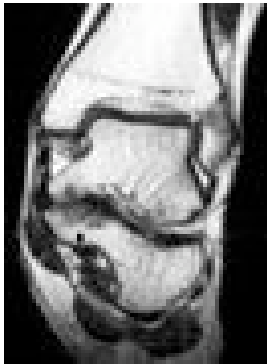


Figure 1. Subtalar joint axis

Often reported as a single axis, a helical screw axis and a bundle of axes, the subtalar joint is responsible for several movements about the ankle; inversion and eversion in the transverse plane; plantar flexion and dorsiflexion in the sagittal plane; and adduction and abduction in the frontal plane. “Subtalar inversion helps to bring about stability of the foot during single-limb stance.” (Backus and Sherry, 1999) Subtalar eversion occurs as a mechanism of shock absorption when the foot makes contact with a surface. Five muscles help control inversion of the subtalar joint axis and cross the medial side of the joint: tibialis posterior, tibialis anterior, flexor digitorum longus, flexor hallucis, and soleus. Four muscles are responsible for eversion of the subtalar joint axis: extensor digitorum longus, peroneus tertius, peroneus longus, and peroneus brevis (figure 2).



Figure 2. Muscles that cross the subtalar joint axis.

It is the motion at this joint that permits the foot to adapt to a variety of surfaces (Wright et al., 1964). “In addition there is substantial variability in the orientation of this axis in normal individuals; thus the relative motions will also vary among normal individuals.” (Backus and Sherry, 1999) The subtalar joint axis has a wide accepted range of deviation and inclination. The range of deviation is 4 degrees to 47 degrees with

a standard deviation of 11 degrees and a mean deviation of 23 degrees. Subtalar inclination ranges from 20.5 degrees to 68.5 degrees with a standard deviation of 9 degrees and mean range being 42 degrees. (Isman and Inman, 1969)

Ground Reaction Forces

Ground reaction force (GRF) is any external reaction force, specifically one applied by the ground. Ground reaction force is equal in magnitude and opposite in direction to the force that the body exerts on the supporting surface through the foot. GRFs can be represented by Newton 3rd law of motion, action reaction pair.

Several studies have viewed and reported increased pressure under the forefoot with an increase in heel heights in women wearing high-heeled shoes. However few studies have measured GRFs. In a study done by Opila-Correla, K.A. (1990), no significant differences in GRFs were found between younger and older wearers or between experienced and inexperienced high heeled shoe wearers. Another study, measured vertical, anteroposterior and mediolateral direction of GRFs of a women's gait in three different heel heights. The study showed an increase in vertical, anteroposterior and mediolateral ground reaction forces with increased heel height. The highest heel height of 7.62 cm showed a pronounced inflection point compared to those of the lower heels and medium heels tested for vertical GRFs. Anteroposterior and mediolateral GRF appeared later in the stance and support phases for the higher heel height, but did show a significant increase.

Muscular Activity

In high heel gait and standing, many muscles located in the lower extremities and the back are overly worked due to the plantar flexion of the foot. Muscles are at their peak for force generation when they are at resting length. When muscle length increases or decreases beyond its resting length, muscle force production decreases in a bell shaped form. This relationship is seen in high heel wearers. When the heel is raised, as in wearing high-heeled shoes, muscles fibers that innervate the muscles along the leg are shorten. The shortened muscles are now inconsistent with its resting length-tension relation resulting in less force production. Esenyel et al. (2001), found "... the exaggerated plantar flexed position of the ankle joint places the gastro-soleus muscle at a shortened and thus less favorable position on its muscle length-tension curve. Under such conditions, the plantar flexion musculature is in a less advantageous position for power and work generation and consequently less propulsive abilities. (Esenyel et al., 2001)

METHODOLOGY

Five female subjects between the ages of 18-24 years of age were recruited from the local Pennsylvania State University community for this study. Their age, height, weight and shoe size were recorded. The subjects reported not having any musculoskeletal or neuromuscular abnormalities that restrict the range of lower extremity motion, which might make the wearing of high-heeled shoes painful. All subjects were experienced wearers of high-heeled shoes as evidenced by self-reported wearing usage of at least twice per week. These criteria were established to control variation among subjects in their motor control that could result in differences in habitual versus sporadic high-heeled shoe wearers. All methods were in accordance with the guidelines set forth by the Human Subjects Review Board of The Pennsylvania State University, University Park, Pennsylvania.

Two different shoes (figure 3) were used in this investigation. The first shoe, a flat open toed shoe, had an average heel height of 1 cm and a stacked block heel. The second shoe was a high-heeled open toed shoe with an average heel height of 2.5 cm with a stiletto-spiked heel. Both shoes were commercially available and purchased through a Payless Shoe manufacturer. The shoes were primarily chosen due to their similarity of construction at the forefoot and their access to the calcaneus so that the main difference between shoe models was the height and type of heel. Shoe sizes ranged from 7, 7.5, 8, 8.5, to 9.



Figure 3 Shoes used in the study. Shoe to the left, the flat, measures with a 1" heel. Shoe on the right, the high heel, measures a heel height of 2.5".

Kinetic data of ground reaction force as well as the point of application of that force was collected with a Kistler Instrument. Corporation force plate mounted flush to the floor. Kinematic data was collected simultaneously with the kinetic data using a Video-based motion analysis system. An Eagle system (Motion Analysis Corporation.) that consisted of six video cameras tracked the locations of spherical reflective markers in three dimensions. Electromyographical activity was collected using a telemetered EMG system (Noraxon Corporation.). Recording electrodes were connected to a battery powered transmitting unit worn on the subject's belt.

Determination of the subtalar joint axis was the first procedure that the subject undertook. Participants were asked to sit at the edge of a table to perform a non-invasive technique for location of the subtalar joint. The applications of eight, 9-mm-diameter reflective markers, using double sided tape were applied to the skin overlying the anterolateral aspect of the tibia and the lateral aspect of the calcaneus of the right leg on each subject. Four markers each were applied to the anterolateral tibia and the lateral aspect of the calcaneus in an asymmetrically box fashion. This was done to help increase

the tracking of the cameras. Quiet standing of three seconds was done to record the location of the reflective markers to serve as a template for later tracking the location of the subtalar joint axis. Palpitation of six anatomical landmarks: the right lateral malleolus, right medial malleolus, right lateral tibial condyle, right medial tibial condyle, right foot heel, and the right second metatarsal head were done.

An additional 9-mm-diameter reflective markers were placed on these locations. In addition, markers were placed to help computerize the structure of the shank and foot. Three reflective markers were placed on the ground to help digitize the plane coordinates of the foot. Subjects were asked to stand for three seconds of quiet standing while the cameras recorded the location of the 17 markers (14 markers on the body and three on the ground). Participants then had the additional markers from the right lateral malleolus, right medial malleolus, right lateral tibial condyle, right medial tibial condyle, right foot heel, right second metatarsal head removed and the three placed on the ground to leave the remaining eight markers placed on the shank and calcaneus.

Participants were affixed to a Marionette System (figure 4) on the table so that feet were dangling. The participant's thigh was stabilized with a velcro strap and the foot was put into dorsiflexion by pulling up on the Marionette System as the forefoot is hanging from a platform by a velcro strap. Alternately pulling on the strings tension to produce a slow full side-to-side rocking motion of the foot was done to provide full usage of the subtalar joint axis. The motions of the eight markers were tracked using the Eagle system (Motion Analysis Corporation.) video-based motion analysis system. Ten 30-second trails were recorded for each to ensure accuracy.

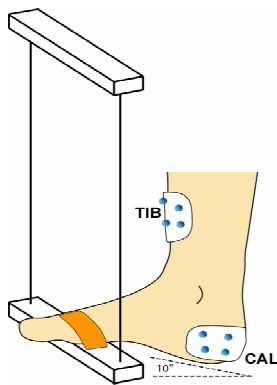


Figure 4. Non-invasive subtalar joint axis determination. The foot is moved about the subtalar axis and three-dimensional motion of the heel and shank is recorded.

Electromyographical activity of the tibialis anterior, peroneals, lateral gastrocnemius and medial gastrocnemius muscles were collected using surface electrodes. Skin preparation consisted of treating appropriate areas with alcohol swabs to ensure dead skin removal and increase EMG activity readings. Self-adhesive circular Ag/AgCl electrodes of 1-cm diameter were placed approximately 2 cm apart in the middle of the muscle bellies in an attempt to minimize cross talk and remain in the same placement for data collection of all shoes.

For each subject the order of the shoe condition was randomly assigned and three standing trails for five seconds each were collected for each condition. Subjects stood still in a normal relaxed position on a wooden platform two inches above the floor. (figure 5) A cutout in the platform contained a wooden block that rested atop the force plate set in the floor in order to collect heel ground reaction force. The platform itself was supported by the floor and not the force plate. Subjects placed just the heel of the right shoe atop the wooden block.



Figure 5. Subject standing in high heels on platform over force plate

Lastly participants performed trails to elicit EMG percent maximal. Two trails for each muscle will be performed for five seconds each. For tibialis anterior subjects were asked to first walk on the outer foot borders for five seconds and then invert against manual resistance applied by the investigator. For the peroneal muscles, participants were asked to walk on the inner border of the foot for one trail and then evert against manual resistance applied by the investigator for the last trail. The participant was then asked to push against a wall using maximum force and then perform a resistant toe rise for trails to elicit percent maximum of EMG activity in the gastrocnemius muscle.

A two-way repeated measure, ANOVA, using Sigma Stat software was used to compare the biomechanical variables (kinematic, kinetic and EMG) between shoe conditions. When a significant difference was found, a Tukey post hoc analysis was performed. The level of significance was chosen as $p < 0.05$.

RESULTS

A one-way analysis of variance test (ANOVA) with repeated measures was performed to determine the effect of shoe condition on subtalar joint moment and activity in the tibialis anterior, peroneals, lateral gastrocnemius and medial gastrocnemius muscles for each subject.

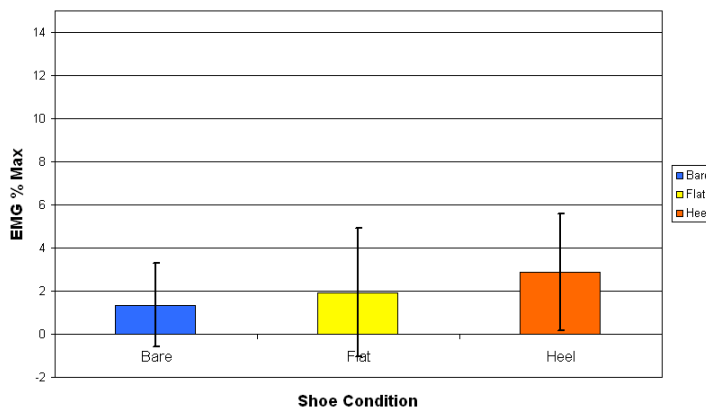
Mean and standard deviation values of activity of each muscle considered for the shoe conditions of bare, flats and high heels are shown in table 1. There were no significant differences ($p < 0.050$) shown for muscle EMG for the tibialis anterior ($p = 0.883$), peroneals ($p = 0.077$), lateral gastrocnemius ($p = 0.093$) or medial gastrocnemius ($p = 0.330$).

ANOVA testing did reveal significant differences in subtalar joint moment between treatments ($p = 0.006$). Post-hoc mean comparisons (Tukey) were calculated for the factor of each shoe condition heel versus flat, heel versus bare and bare versus flat (table.2). Comparison showed the high-heeled condition to be different from both the bare and flat conditions with a significant difference of heel versus flat ($p = .006$) and heel versus bare ($p = 0.028$). No significant difference in mean value for subtalar joint moment was found for bare versus flat condition (0.501).

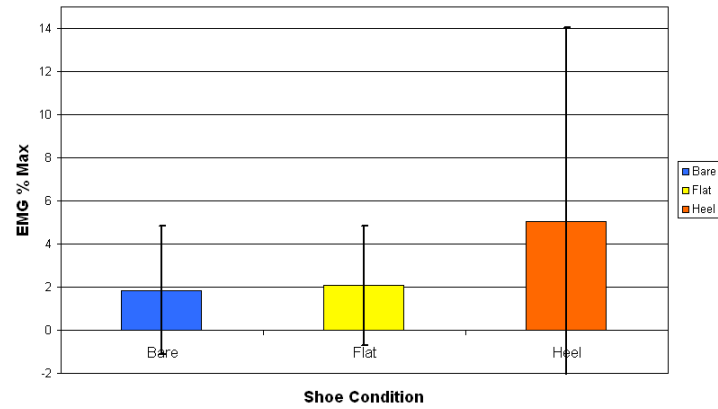
Tibialis Anterior EMG



Peroneals EMG



Medial Gastrocnemius EMG



Lateral Gastrocnemius EMG

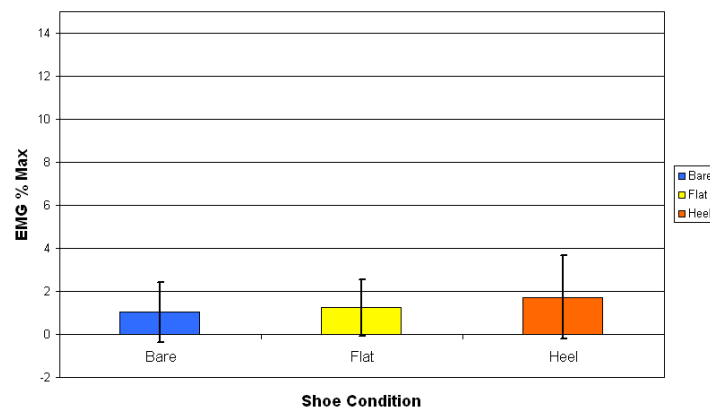


Table.1 - Mean and standard deviation of EMG activity of the tibialis anterior, peroneals, medial gastrocnemius and lateral gastrocnemius for each shoe condition.

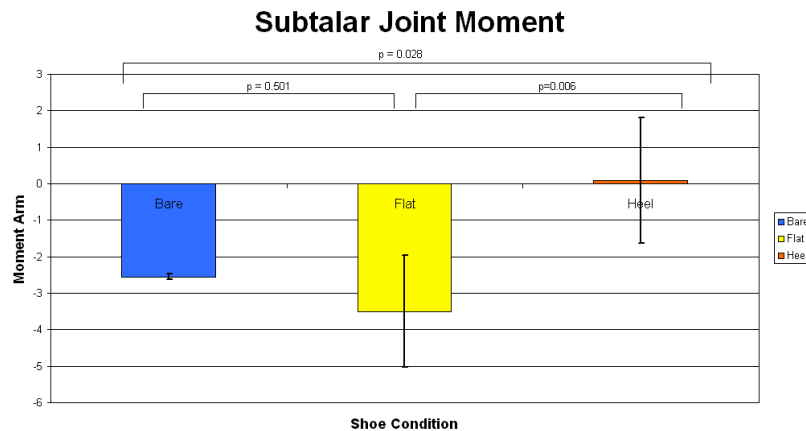


Table 2- Mean, standard deviation and post hoc statically p values of subtalar joint moments for shoe condition bare, flat and high heeled.

DISCUSSION

The study is purpose was to determine the action of the ground reaction force upon the heels of women standing in high-heeled shoes by looking at the anatomical difference about the subtalar joint axis. It was to be determined where the ground reaction force acts relative to the subtalar joint axis and whether muscle activity while standing depends on this result.

Subtalar joint axis orientation was consistent with ranges reported by Isman and Inman (1969) with inclination ranges being within 20.5 degrees to 68.5 degrees and deviation ranges being within 4 degrees to 47degrees. Location of the subtalar joint axis had a fairly good repeatability from trial to trial within subjects. For example Subject #6 had only a four-degree standard deviation for both deviation angle and inclination angle. Subtalar joint axis orientation and repeatability could be due to the upward pull and placing the foot into a dorsiflexed position. This position locks that talocal joint so that the subtalar joint axis is solely responsible for the side-to-side rocking motion about the ankle. Other non- invasive techniques to validate location of the subtalar joint axis are being tested and on the way.

Moments about the subtalar joint axis were significantly different. Significant difference ($p=0.006$) was found with comparison to high heels in the conditions of heel versus flats shoes ($p=0.006$) and heel versus barefoot ($p=0.028$). In general, while standing in high-heeled shoes women experience a small positive moment about the

subtalar joint axis at the ankle. This positive moment causes an inverting torque experienced by women standing in high-heeled shoes. During barefoot and flat shoe conditions, a negative moment was seen resulting in a larger everting torque about the ankle.

All differences in EMG activity for the tibialis anterior, peroneals, lateral gastrocnemius and medial gastrocnemius were insignificant. However, two trends were seen in muscle activity by women while standing in high-heeled shoes. Similar to other studies, the first trend was that there was more muscle EMG activity of the tibialis anterior peroneals lateral gastrocnemius and medial gastrocnemius with increasing heel height. More specifically, a second trend was seen toward more activity in the high-heeled shoe condition for the peroneals and lateral gastrocnemius muscles. Both muscles showed close to significant results with the peroneals having $p=0.007$ and lateral gastrocnemius having a $p=0.093$.

Both the lateral gastrocnemius and peroneals are muscles that help eversion of the heel. The increase of activity of these muscles could have been a compensating response for the more positive- inverting moments of the ground reaction force while wearing high-heeled shoes. An increase in peroneal activity is similar to the data presented by Stefanyshyn et al., (2000). The increased activity of the peroneal muscle could be due to the controlling of the increased plantarflexion of the foot when standing in high heels. Another response to the increased activity of the peroneal is its role of protection to the foot from sudden inversion about the ankle and a stabilizer. The increased activity could be a required response, to stabilize the ankle joint when wearing high heels.

The response of the lateral gastrocnemius activity was similar to one other study conducted by Gefen et al. (2002), which showed the two heads of the gastrocnemius (lateral head and medial head) to respond differently when wearing high-heeled shoes. Other studies agree with no significant differences in EMG activity of the gastrocnemius (Stefanyshyn et al., 2000; Ono, 1969). While other studies showed a progressive negative linear relationship with increasing heel heights for EMG activity of the gastrocnemius (Lee et al., 1990). The explanation as to why a trend of activity of lateral gastrocnemius increased more in high heels versus that of the medial gastrocnemius is that the lateral head may act more intensively to produce the forces required to compensate the positive moment of the ground reaction forces. As seen in previous study resultant forces generated by the lateral gastrocnemius are transferred down to the Achilles tendon to the calcaneus. Since the foot is plantar flexed, the Achilles tendon is slackened and the lateral gastrocnemius helps to take up this slack, which could result in an inverting moment that acted to incline the foot's skeleton laterally. (Gefen et al., 2002)

EMG activity of the tibialis anterior was contradictory with results of some other studies. Studies conducted by Joseph (1968) and Lee et al. (1990) showed a decrease in EMG activity while standing in high heeled shoes. The tibialis anterior helps with dorsiflexion of the foot and acts as a stabilizer of the ankle. This study concluded while not significant a trend of increase muscle activity was seen in high heels for all muscles. Since the subjects were experienced high heel wearers they may no longer experience

feelings of instability when wearing high heels. The increased EMG activity by the peroneals and lateral gastrocnemius seen to compensate for the inverting moment of standing in high heels could possibly not be seen with the tibialis anterior due to its location on the anterior aspect of the shank.

Limitations

Several limitations could have led to the results of this study. All five subjects were college-aged women from the State College, Pennsylvania area. Subjects were also excluded if they did not wear a shoe size from 7, 7.5, 8, 8.5, to 9 comfortably since these were the shoe sizes provided by the researcher. Since the shoes were provided for the subject, new and unfamiliar shoes could have caused a limitation to the study. While EMG activities for all four muscles were insignificant a greater sample size could result in more significant results. Occasional burst of EMG amplitude potential from muscle of some subject while standing in high heels due to instability of posture was seen. Basmajian and Bentzon (1954) also found similar burst of activity in muscles accompanied by women wearing high heels. Placement of Eagle camera could also cause a limitation to this study. Eagle cameras were having difficulty tracking the motion of the marker located on the anteromedial aspect of the tibia and the lateral aspect of the calcaneus. Closer and more precise location of the camera could result in better accuracy of the subtalar joint axis location.

Future Research

Future research will include refining this study to test a greater sample size and to redefine the non-invasive method of the subtalar joint axis location to gather more precise data for analysis. Other future plans are to test shoes with greater heel height than 2.5 cm to look at their effects and to look at the results of ground reaction forces about the subtalar joint axis in an individualized way from subject to subject. Future goals are to test a design of a biomechanical correct high heel shoe that accounts for anatomical differences of the joint axis.

Conclusions

Subtalar joint axis orientations were consistent with ranges reported by Isman and Inman, (1969) and subtalar joint axis location had fairly good repeatability. Moments about the subtalar joint axis were significantly different between the shoe conditions heel versus flat shoes, and between heels versus barefoot. High-heel shoes generally had a small positive inverting moment, but there was a larger everting moment in the barefoot and flat conditions seen. All differences in EMG activity for the tibialis anterior, peroneals, lateral gastrocnemius and medial gastrocnemius were insignificant, but with a trend toward more activity in the high-heeled condition for muscles that evert the heel (peroneals and lateral gastrocnemius). These muscles may have been compensating for the more inverting moments of the ground reaction force while wearing high-heeled shoes.

REFERENCES

- Backus, J., Sherry, 1999. Disorders of the Heel, Rearfoot, and Ankle. 11-13. New York: Harcourt Brace & Company.
- Basmajian, J.V., Bentzon, J.W., 1954. An electromyographic study of certain muscles of the leg and foot in the standing position. Surg. Gynec. Obster. 98, 662-666.
- Esenyel, M., Gitter, A., Walden, G., 2001. Altered Work Distribution in The lower Extremity While Walking in High Heeled shoes. Journal of Biomechanics 29, 405-413.
- Gefen, A., Megido-Ravid, M., Itzchak, Y., Arcan, M., 2002. Analysis of muscular fatigue and foot stability during high heeled gait. Gait and Posture 15, 56-63.
- Harris, G., 2003, 3 December. "If Shoe Won't Fit, Fix the Foot? Popular Surgery Raises Concern.". The New York Times.
- Isman, R.E., Inman, V.T., 1969. Anthropometric studies of the human foot and ankle. Bulletin of Prosthetics Research, 97-129
- Joseph, J., 1968. Pattern of activity of some muscles in women walking on high heels. Annals of Physical Medicine 9, 295-292.
- Lee, CM., Jeong, EH., Freivalds, A., 2001. Biomechanical effects of wearing high-heeled shoes. International Journal of Industrial Ergonomics 2, 321-326.
- Lee, K.H., Matteliano, A., Medige, J., Smiehorowski, T., 1990. Electromyographic Changes of Leg Muscles with Heel Lifts in Women: Therapeutic Implications. Archives of Physical Medicine and Rehabilitation 71, 31-33.
- Linder, M., Saltzman, C.L., 1998. A history of medical scientists on high heels. International Journal of Health Service 28 (2), 201-225.
- Ono, H., 1969. Heel Height and Muscle Activity. Journal of Japanese Orthopedic Association 43, 527-547.
- Opila-Correia, K.A., 1990. Kinematics of high heeled gait. Archives of Physical Medicine and Rehabilitation 71, 304-309.
- Schwartz, R.P., Heath, A.L., 1959. A qualitative analysis of recorder variable in the walking pattern of normal adults. Journal of Bone and Joint Surgery 41, 1065.
- Spraggins, R.E., 2000. A Census Brief entitled Women in the United States: A Profile. Current Population Reports.

- Stefanyshyn, D.J., Nigg, B.M., Fisher, V., O'Flynn, B., Liu, W., 2000. The Influence of High Heeled Shoes on Kinematics, Kinetics and Muscle EMG of Normal Female Gait. Journal of Applied Biomechanics 16, 309-319.
- Stephens, M.M., 1992. Heel Pain- Shoes, Exertion, and Haglunds Deformity. Physician Sportsmedicine 20 (4).
- The Gallup Organization, Incorporation, 1986. "Women's Attitude on Usage of High Heel Shoes."
- Wright D.G., Desai S.M., Henderson W.H., 1964. Action of the subtalar and ankle-joint complex during stance phase of walking. Journal of Bone Joint Surgery AM. 46, 361-366.

Dispersal Patterns of Male White-tailed Deer in Centre County, PA

Milton G. Newberry, III, Penn State
Duane R. Diefenbach, Ph.D.
U.S. Geological Survey
Adjunct Associate Professor of Wildlife Ecology
Penn State
Eric Long, Graduate Research Assistant
School of Forest Resources
Penn State

Abstract: Dispersal direction of male white-tailed deer (*Odocoileus virginianus*) has been hypothesized to be influenced by landscape features such as ridges and valleys. We monitored the dispersal distances and directions of 36 yearling males in Centre County during spring dispersal periods 2002-2004 and fall dispersal periods 2002-2003 using radio telemetry. We hypothesized that the dispersal direction of deer would be bimodal and parallel to the ridge lines in the study area ($\mu_b = 66^\circ$, $\mu_l = 246^\circ$). We found no difference in direction between spring and fall dispersal periods, and no difference between years. The mean angles ($\mu_l = 89$, 95% CI = 71-109; $\mu_b = 269$, 95% CI = 251-289) were different from the direction of the ridgelines. Thus, we concluded that yearling male dispersal is directed but not parallel to the ridges and valleys of Centre County. We recommend future research investigate whether habitat features, natural and man-made barriers, and response to human contact influences dispersal direction.

Introduction

Dispersal among animals is common and is often considered a means to minimize inbreeding (Shield 1987) and serve as a mechanism for establishing new populations or facilitating species range expansion. However, wildlife managers typically ignore dispersal, assume it to be nonexistent, or assume that immigration and emigration are equal (Rosenberry et al. 1999). Mammalian dispersal is typically male biased to avoid inbreeding (Greenwood 1980) and dispersal is most prevalent among yearling bucks in white-tailed deer (*Odocoileus virginianus*, Marchinton and Hirth 1984). Social pressures have been hypothesized to prompt natal dispersal of yearling male white-tailed deer during the breeding season (i.e., August to November) or during the fawning season (i.e., May to June). For example, sexual competition with other males (Kammermeyer and Marchinton 1976) and aggression from a yearling male's mother (Holzenbein and Marchinton 1992a) or related adult females (Ozoga and Verme 1985) has been implicated as potential proximate mechanisms of dispersal. Inbreeding avoidance, as exemplified by

maternal antagonism, has been shown to influence dispersal rates of orphan and non-orphan juvenile males, such that orphaned males are less likely to disperse than non-orphans (Holzenbein and Marchinton 1992). For white-tailed deer, natal dispersal by yearling males represents a potentially significant exchange of individuals across areas. Yearling male dispersal can lead to changes in population sizes (Hawkins et al. 1971) and sex ratios (Kammermeyer and Marchinton 1976, Marchinton 1984, Rosenberry et al. 1999).

White-tailed deer typically disperse 8-12 kilometers but movements >200 kilometers have been reported (Kernohan et al. 1994). Some studies have shown that deer movements are directional and associated with watersheds (Sparrowe and Springer 1970). Meanwhile, according to Dusek et al. (1989), dispersal was bimodal on roughly a northeast-southwest axis that conformed to that of the Lower Yellowstone River valley, whereas other studies have shown no relation to dispersal and physiographic features (Verme 1973, Kilgo et al. 1996).

Understanding dispersal patterns is important for effective management of deer (Miller et al. 1995), and dispersal data can help assist in the prediction of movement and spread of diseases. Diseases, such as chronic wasting disease (CWD), have presented problems for many states in the Midwest. Chronic wasting disease is fatal in white-tailed deer, mule deer (*O. hemionus*), and elk (*Cervus elaphus*) and is caused by transmissible protease-resistant prion proteins (Joly et al. 2003). Current epidemiological models suggest that CWD is lethal and presence of the disease can result in complete extirpation of infected populations (Miller et al. 2000). Knowledge of dispersal movements would be helpful when formulating strategies to control or minimize the spread of CWD.

Centre County has long linear ridges and valleys that could potentially influence dispersal direction. The distinct division between the ridges and valleys provide habitat preferred by deer because of the juxtaposition of forested and field vegetative types. According to Hiller (1996), the edge of forests and agricultural fields supply a wide diversity of food, shelter, and habitat components for deer. Centre County is an appropriate site for this study on dispersal direction. The long linear ridges in the county provide a means to examine potential topographic influences on dispersal direction. Our objective was to capture yearling bucks and monitor their dispersal to test whether the topography of Centre County, Pennsylvania, was related to dispersal direction.

Study Area

This study was conducted in Centre County, Pennsylvania. Centre County (2,870 km²) spans both the Allegheny Plateau and the Ridge and Valley region of Pennsylvania. Our study area in southern Centre County lies within the Ridge and Valley region. Unlike northern Centre County, the southern region is primarily private land, though some public lands exist primarily on the ridges. Ridges are primarily forested with mixed or hardwood tree species and oriented in a northeast (66°) - southwest (246°) direction. The Centre County area represents a landscape with both agricultural and heavily wooded areas. Deer densities range from 21 - 43 deer per square mile of land (Pennsylvania Game Commission 2003). Common tree species on the study area include northern red oak (*Quercus rubra*), white oak (*Quercus alba*), red maple (*Acer rubrum*), white pine (*Pinus strobus*), and yellow poplar (*Liriodendron tulipifera*).

Methods

Capture and Data Collection

We captured yearling males 6-9 months of age using drop nets, Clover traps, and rocket nets (Bookhout 1994). Upon capture, deer were masked and sedated (Rosenberry et al. 1999). We tagged each ear with two uniquely numbered ear tags, which were labeled with contact information. Each deer was then fitted with a VHF-transmitter or Global Positioning System (GPS) collar.

Each deer was monitored and located 1-3 times per week using biangulation of signal bearings from >1 known locations. We obtained more signal bearings from additional locations if radio signals were disrupted by radio traffic, electrical interference from power lines, and signal bounce created by the topography of the study area. Aerial telemetry was used to locate deer that dispersed long distances and could not be located from the ground. Location data were entered into LOAS 2.04 and plotted on a map of Centre County.

We defined dispersal as the movement of an individual from its birth home range to the place where it reproduces or would have reproduced if it had survived and found a mate (Nelson 1993). Dispersal movements are long-distance movements without return to previously established home ranges and occur in most white-tailed deer populations (Kernohan et al. 1994). Dispersal occurs during spring and fall; we defined spring dispersal as May to the end of June and fall dispersal as September to November. For bucks that did not disperse, adult home range formation was defined to have begun 1 November.

Data Analysis

We calculated mean natal and adult locations for each buck. Dispersal distance and direction were the distance and direction from the mean natal locations to the mean adult locations. We consulted several other studies to choose a minimum value of dispersal distance. Nelson (1993) stated that movements <4 km were recorded as 0 dispersal distance. According to Kilgo et al. (1996) and Rosenberry et al. (2001), dispersal was classified as a movement ≥ 2 kilometers from the natal range. Therefore, we classified a buck as a disperser if it moved ≥ 3 km from its natal home range because this criterion was used in other studies.

The Multi Response Permutation Procedure (MRPP; Cade and Richards 2001), using the ARC option for directional data, was used to test for yearly and seasonal differences in the dispersal directions for the sample. We used the V test (Batschelet 1981) (Figures 1, 2a, 2b) to test whether dispersal direction of the yearling bucks was uniform or directed. We define:

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n \cos(2 \theta_i)$$

$$\bar{y} = \frac{1}{n} \sum_{i=1}^n \sin(2 \theta_i)$$

$$r = \sqrt{\bar{x}^2 + \bar{y}^2}$$

$$\bar{\theta} = \begin{cases} \arctan\left(\frac{\bar{y}}{\bar{x}}\right) & \text{if } \bar{x} > 0 \\ 2\pi + \arctan\left(\frac{\bar{y}}{\bar{x}}\right) & \text{if } \bar{x} < 0 \\ \pi/2 & \text{if } \bar{x} = 0 \text{ and } \bar{y} > 0 \\ 3\pi/2 & \text{if } \bar{x} = 0 \text{ and } \bar{y} < 0 \end{cases}$$

where n = number of juvenile males who dispersed greater than 3 km and $\bar{\theta}$ is the direction the animal dispersed in radians, and

$$V = r \cos(\bar{\theta} - 2\theta_0),$$

where $\theta_0 = 66^\circ$ is the direction of the ridges. We then used the test statistic U to test for uniformity of direction:

$$U = V \sqrt{2n}.$$

If dispersal were directional based on the V test, we calculated mean dispersal angles and 95% confidence intervals using 999 bootstrapped samples. If 95% CIs overlapped the direction of the ridges ($\theta_0 = 66^\circ$, $\theta_1 = 246^\circ$) we concluded that we had no evidence that dispersal direction was not parallel to the ridges.

Results

We captured 140 yearling bucks and located them by radio telemetry over 3 years. Of these, 50 were censored because of lack of location data for either natal or adult ranges because of deer mortality or failed transmitters. Fifty-four deer were not included in the analyses either because they did not disperse or apparent dispersal movements were <3 km. The remaining bucks ($n = 36$) provided dispersal data that matched our inclusion criteria. Dispersal distance ranged from 3.1 km to 21 km and the mean dispersal distance was 7.8 km. We found no difference in dispersal direction between years and seasons (MRPP test, $P > 0.10$). Therefore, we pooled data from all years and seasons. The V test for uniformity of direction was rejected ($U = 2.17$; $P < 0.05$). The mean angles ($\theta_1 = 89^\circ$, 95% CI = 71-109; $\theta_2 = 269^\circ$, 95% CI = 251-289) were different from the direction of the ridgelines (Figure 3).

Discussion

The results indicate that deer dispersal was directed, and dispersal directions were similar to ridges and valleys in Centre County. However, 95% CIs of the dispersal direction did not overlap the exact angles of the ridges and valleys. The ridges and valleys align with a northeast-southwest direction; however, average dispersal direction of bucks was approximately east-west. We have no explanation for the direction of dispersal that we observed.

One possible explanation is the presence of quality habitat. The ridges and valleys in Centre County along with agricultural clearings create edge habitat appropriate for deer. This edge provides a wide diversity of food, shelter, and habitat components (Hiller 1996). Deer prefer areas where two vegetative types such as woods and fields or grasslands and brush come together such as in the ridges and valleys of Centre County. This edge habitat is encountered when moving east or west along the ridges and valleys. As a conceivable result, the male juveniles are dispersing in these directions. However, in one study conducted by Kilgo (1992), influences on animal movements such as food quality, water, and refugia possibly affected the movements of a few individuals in Florida, USA, whereas in another study conducted by Kilgo et al. (1996), food and habitat did not offer satisfactory explanations to why dispersal was in an east-west direction. Food availability becomes a possible factor in influencing dispersal because it differentiates from spring dispersal period to fall dispersal period and can potentially cause the juvenile bucks to disperse across the ridges and valleys. Further research is needed to determine whether habitat quality and food availability affect dispersal direction.

Another factor that can be influencing dispersal is seasonally changing habitat. When cover conditions were optimum, as in summer, deer movements tend to be limited (Sparrowe and Springer 1970). When cover is scarce, deer movements are partly controlled by available heavy cover. According to Sparrowe and Springer (1970), deer often crossed wide expanses of open fields between patches of heavy cover in eastern South Dakota. Juvenile males are possibly dispersing wherever there is heavy cover on the ridges and valleys during the spring dispersal, whereas, during the fall dispersal, essential cover becomes limited. This limitation potentially affects dispersal direction during the fall dispersal period. Many deciduous forests within the study area become bare, whereas patches of evergreen trees are visible on ridges and valleys. These patches of evergreen trees can pose as a corridor for dispersal during the fall dispersal period, assuming these patches are large enough for long distance dispersal. Research must be conducted to test and compare dispersal direction between dispersal periods where habitat is changing seasonally.

Long distance dispersal may also be directed due to barriers. The valleys in Centre County contain farms and residential areas. These areas include human interaction, possible encounters with dogs, and car collisions, which are a major cause of white-tailed deer fatalities. Major highways, together with agricultural and residential areas, may represent barriers to deer dispersal, and juvenile males at the edge of the ridges and valleys may disperse east or west to avoid human contact. Movements in any other direction, including our hypothesized directions, require the deer to cross major

highways such as I-80. The presence of cars may possibly induce juvenile males to disperse parallel to major highways. Additional research is needed to test whether roadways pose as a barrier to deer and may cause directional dispersal.

According to Dusek et al. (1989), juvenile male white-tailed deer dispersed along the river bottom of the Lower Yellowstone River. Twenty-seven of 33 dispersing deer did not cross the river itself but had a tendency to remain in the river bottom. Waterways such as rivers and streams can be potential natural barriers. However, in our study area, deer did disperse across the areas where Penns and Bald Eagle Creeks lie, suggesting these smaller streams may not be major factors influencing dispersal direction.

Future research can be conducted to investigate whether other factors influence dispersal direction. Possible factors include habitat features, natural and man-made barriers, and human contact.

Acknowledgments

Milton Newberry would like to thank Wendy Vreeland for showing me how to use the radio telemetry equipment effectively and allowing me to accompany her in the field when locating bucks. Milton Newberry would like to thank Curtis Price, Teresa Tassotti, the McNair Scholar Program and staff, and fellow scholars for helping me through this long process. Milton Newberry would also like to thank his family and friends for giving him the strength to be successful in college.

WORKS CITED

- Batschelet, E. 1981. *Circular statistics in Biology*. Academic Press, London, U.K.
- Bookhout, T. A. 1994. *Research and Management Techniques for Wildlife and Habitats*. The Wildlife Society. Bethesda, Maryland, USA.
- Cade, B. S, and J. D. Richards. 2001. Fort Collins, CO: U.S. Geological Survey, Midcontinent Ecological Science Center. 107 pp.
- Dobson, F. S. 1982. Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour* 30:1183-1192.
- Dusek, G. L., R. J. Mackie, J. D. Herriges, and B. B. Compton. 1989. Population ecology of white-tailed deer along the lower Yellowstone River. *Wildlife Monographs* 104.
- Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behavior* 28:1140-1162.
- Hawkins, R. E., W. D. Klimstra, and D.C. Autry. 1971. Dispersal of deer from Crab Orchard National Wildlife Refuge. *Journal of Wildlife Management* 35:216-220.

- Hiller, Ilo. 1996. *The White-tailed Deer*. College Station, TX: Texas A&M University Press.
- Holzenbein, S., and R. L. Marchinton. 1992*a*. Spatial integration of maturing white-tailed deer into the adult population. *Journal of Mammalogy* 73:326-334.
- Holzenbein, S., and R. L. Marchinton. 1992*b*. Emigration and mortality in orphaned male white-tailed deer. *Journal of Wildlife Management* 56:147-153.
- Kammermeyer, K. E. and R. L. Marchinton. 1976. Notes on dispersal of male white-tailed deer. *Journal of Mammalogy* 57:776-778.
- Joly, D.O., Ribic, C.A., Lagenberg, J.A., Beheler K., Batha C.A., Dhuey B.J. et al., Rolley, R. E., Bartelt, G., Van Deelen, T. R., Samuel, M. D. 2003. Chronic Wasting Disease in Free-ranging Wisconsin White-tailed Deer. *Emerging Infectious Diseases* 9:599-601
- Kernohan, B. J., J. A. Jenks, and D. E. Naugle. 1994. Movement patterns of white-tailed deer at Sand Lake National Wildlife Refuge in South Dakota. *Prairie Naturalist* 26:293-300.
- Kilgo, J. C. 1992. Effects of environmental influences on home rang and activity of adult female white-tailed deer on the Osceola National Forest, Florida. - Final Report to Florida Game and Fresh Water Fish Commissions, Department of Wildlife and Range Sciences, University of Florida, Gainesville, 87 pp.
- Kilgo, J. C., R. F. Labisky, and D. E. Fritzen. 1996. Directional long distance movements by white-tailed deer (*Odocoileus virginianus*) in Florida. *Wildlife Biology* 2:289-292.
- Marchinton, R. L., and D. H. Hirth. 1984. Behavior. Pp. 129-168 *in* L.K. Halls, ed. *White-tailed deer: ecology and management*. Stackpole Books, Harrisburg, PA.
- Miller, K. V., R. L. Marchinton, and J. J. Ozoga. 1995. *Deer Sociobiology*. K.V. Miller and R.L. Marchinton, eds. *Quality whitetails: the why and how of quality deer management*. Stackpole Books, Mechanicsburg, PA.
- Nelson, M. E. 1993. Natal dispersal and gene flow in white-tailed deer in northeastern Minnesota. *Journal of Mammalogy* 74:316-322.
- Nixon, C. M., Hansen, L. P., Brewer, P. A., and Chelsvig, J. E. 1991. Ecology of white-tailed deer in an intensively farmed region of Illinois. *Wildlife Monographs* 18:1-77.

- Ozoga, J. J., and L. J. Verme. 1985. Comparative breeding behavior and performance of yearling vs. prime-age white-tailed bucks. *Journal of Wildlife Management* 49:364-372.
- Pennsylvania Game Commission. 2004. Pennsylvania Hunting and Trapping Digest 2004-2005. Harrisburg, PA
- Pennsylvania Game Commission. 2003. Wildlife Management Annual Report. Job No. 21001: Estimating County Deer Population Sizes & Growth Rates. Harrisburg, PA.
- Porter, W. F. 1991. White-tailed deer in eastern ecosystems: implications for management and research in national parks. United States Department of Interior, National Park Service: Denver, Colorado, USA.
- Rosenberry, C. S., M. C. Conner, and R. A. Lancia. 1999. Population effects of white-tailed deer dispersal. *Wildlife Society Bulletin* 27:858-864.
- Rosenberry, C. S., R. A. Lancia, and M. C. Conner. 2001. Behavior and dispersal of white-tailed deer during the breeding season. *Canadian Journal of Zoology* 79:171-175
- Shields, W. M. 1987. Dispersal and mating systems: investigating their causal connections. Pp. 3-24 *in* B.D. Chepko-Sade and Z.T. Halpin, eds. *Mammalian dispersal patterns: the effects of social structure on population genetics*. University of Chicago Press, Chicago, Illinois.
- Sparrowe, R. D., and P. F. Springer. 1970. Seasonal activity patterns of white-tailed deer in eastern South Dakota. *Journal of Wildlife Management* 34:420-431.
- Verme, L. J. 1973. Movements of white-tailed deer in Upper Michigan. *Journal of Wildlife Management* 37:545-552.

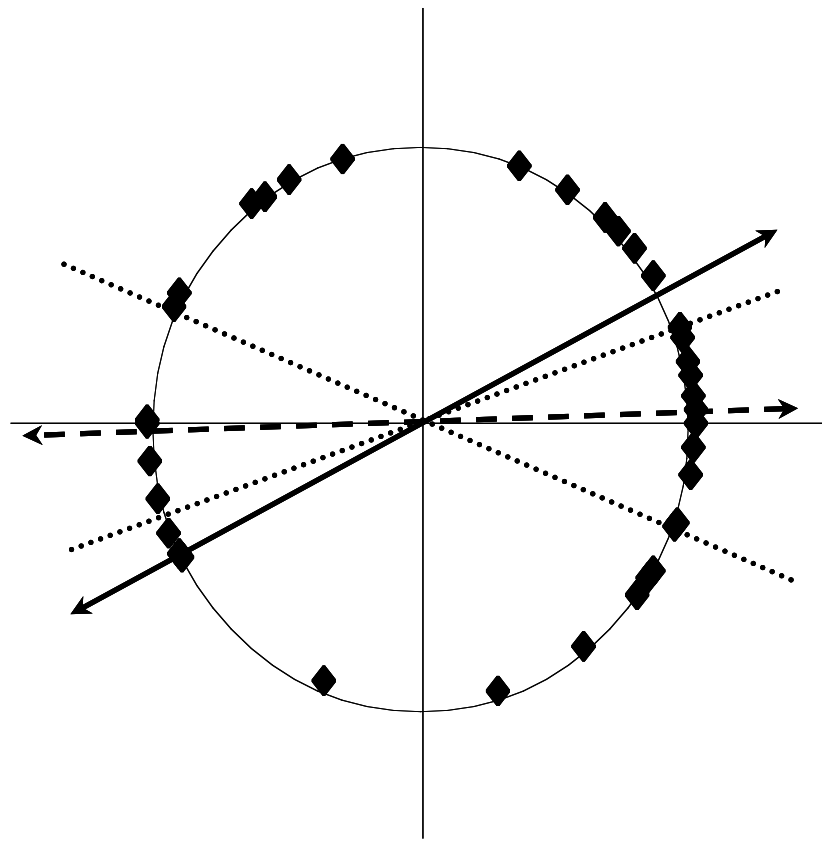


Figure 1. Observed dispersal directions of juvenile male white-tailed deer ($n=36$) in Centre County, PA, 2002-04. 95% confidence intervals (dotted lines) of mean dispersal directions (dashed line) did not overlap hypothesized mean dispersal directions (solid line), which are parallel to ridges and valleys in the study area.

The Role of PPAR- α in Regulating the Symptoms of Allergic Asthma

Tiffany A. Polanco, McNair Scholar, Penn State
Avery August, Ph.D
Associate Professor of Immunology
Veterinary Science Department
Penn State

Abstract

Allergic Asthma is a pulmonary disease that causes difficulty breathing due to airway obstruction, inflammatory infiltrates, and hypersensitivity to allergens and non-specific stimuli. There is no cure, but there are treatments, many of which cause detrimental side-effects. A ligand for the PPAR- α has been shown to have an anti-inflammatory response. We are therefore determining whether the PPAR- α ligand, GW1929, can affect the symptoms of allergic asthma in a murine model of the disease. We discovered that GW1929 does have some therapeutic effects on allergic asthmatic mice. This includes a slightly lower eosinophil infiltration, and a slightly better breathing ability.

Introduction

Allergic asthma is a pulmonary disease that causes difficulty breathing due to airway obstruction, inflammatory infiltrates, and hyper-reactivity to allergens and non-specific stimuli. Twenty-six million Americans have asthma; 8.6 million of those 26 million are under 18. This disease can cause many discomforts in the lives of children and adults. In many children, allergic asthma may cause behavior disorders. Children with this disease have trouble sleeping because the difficulty breathing during the night causes interrupted sleep. As a result, they are fatigued during the day and have trouble concentrating in school and interacting with fellow classmates [1]. In adult life, the disease can be impeding as well. Sleep deprivation is the common factor, but as adults, it causes difficulty concentrating at work and adds unneeded stress to the life of the patient and their family.

There is no cure for the disease, and many treatments today can cause detrimental side effects. Current treatments for allergic asthma may cause conditions such as osteoporosis, candidiasis, Churg-Straus Syndrome or Cushing's syndrome [2]. Osteoporosis, Churg-Straus, and Cushing's are all caused by an excess of glucocorticoid, of which most treatments today are composed [3, 4].

The synthetic molecule GW1929 is a tyrosine based potent agonist of the Peroxisome Proliferator-Activated Receptor- α [5]. GW1929 has a very high affinity for the receptor PPAR- α . This high affinity creates higher potency than natural ligands which don't have this high affinity. PPAR- α is a nuclear receptor and a transcription factor, that is activated by specific ligands. Once the ligand binds the receptor, the receptor is activated, then moves into the nucleus and becomes a transcription factor,

where it influences gene expression. Thus, activation of a target gene transcription depends on a ligand binding to the receptor. PPAR- γ agonists have been proposed to reduce cytokine production by inhibiting pro-inflammatory transcription factors, therefore modulating the inflammatory response [6, 7]. Peroxisome proliferator-activated receptor- γ agonists (ligands) have been shown to be anti-inflammatory and may be able to relieve the symptoms of allergic asthma.

In this report, we use GW1929 to determine whether a PPAR- γ ligand can affect the symptoms of allergic asthma in a murine model of this disease. The mouse is commonly used to model allergic asthma as it mimics many of the properties of the human disease. In addition, there are many immunological reagents and cell markers that are available and have been defined that make it simpler to study the inflammatory cascade in the mouse [8]. We use the mouse as it is a good species to use for measuring inflammatory responses because the immune response is very similar to that of humans [9, 10].

Materials and Methods

Mice – The mice we used were wild type Balb/c mice originally obtained from Jackson Laboratory (Bar Harbor, ME) and bred in the animal facility at Penn State University (PSU). Mice were fed water and food *ad libitum* and cared for according to institution guidelines. The experimental protocol was approved by the IACUC of PSU.

Experimental Design- The experimental design consisted of four groups of mice with five mice per group. Group I was not induced to develop allergic asthma and was not to be treated with the ligand. This was our control group and was used as a reference point to guide our data analysis. Group II was primed for allergic asthma, but was not treated with the ligand. This group was compared to group I (the control group) to determine the difference in lung function when allergic asthma is induced. This information helped us determine how effective the ligand is in relieving symptoms of allergic asthma. The third group (group III) was primed for allergic asthma and treated with the ligand. This group was compared to the control group to determine how close the lung function is to normal. They were also compared to group II to determine how much better the lungs function with the ligand than without. There was also a fourth group. This group (group IV) was not primed to develop allergic asthma and was treated with the ligand. This group was compared to the control group to determine if the ligand causes any side effects.

Priming – Mice were primed with Ovalbumin (OVA) (Sigma, St. Louis, MO) complexed with Imject Alum (Alum) (Pierce, Rockford, IL) in phosphate buffered saline (PBS). We used the Ovalbumin as the antigen or allergen, and the imject alum as an adjuvant. The adjuvant enhances the immune response. The mice were primed with 222 μ g of 10 μ g Ovalbumin and 1mg imject alum solution intra-peritoneally (IP) on days 0 and 5 of the study. Mice that were not to contract the disease were given PBS and imject alum on days 0 and 5.

Challenge – On days 12 to 15 mice that were primed with the OVA/Alum complex were given OVA in PBS solution intra-nasally (IN). This causes a local allergic reaction in the lungs resembling allergic asthma. Mice that were not primed to develop allergic asthma were given PBS intra-nasally on days 12 through 15. Mice primed and challenged experience increased inflammatory cell infiltration into the lung, thickening of epithelial cells in the bronchioles of the lung, mucus secretion, and increases in IgE in the serum [11]. These are hallmark symptoms of allergic asthma.

Treatment - Each mouse received 1 μ g of .5 μ g GW1929 and .5 μ g PBS intra-peritoneally per day on days 12 through 15. The mice that did not receive the ligand were given 1 μ g PBS on these days.

Collection of Data - Data was collected using a Buxco whole body plethysmograph on day 16. This machine measures lung function by calculating pressure changes inside a chamber where the live mouse is held. The machine is able to accurately distinguish between applicable breaths and breaths when the animal is just sniffing around. When the animal has made a certain number of applicable breaths the machine averages these breaths and tells us the average volume of air inspired and expired. The animal is exposed to different concentrations of aerosolized methylcholine in the chamber to gauge the reactions to the stimulant. A mouse without allergic asthma will have a weaker reaction to the methylcholine than one with allergic asthma. Bronchial responsiveness to methylcholine relates closely to the presence and severity of asthma [12]. Analyzing each mouse in the plethysmograph can take up to an hour per mouse. Since there were five mice to each group, we measured one group per day, staggering the experiment by staggering the priming and treatment. The plethysmograph was calibrated before testing each group of mice.

Histology – One lung from each mouse was harvested and fixed in formaldehyde on day 16. The lungs were embedded and sectioned to be stained with H&E for analysis. The slides were analyzed and graded by severity of the disease on a scale from one to four. Sections of the lung that were considered average for each group are displayed and discussed below.

Determination of eosinophils in lungs- For each mouse, one lung was harvested and dissociated using collagenase (150U/ml). These populations were analyzed using an Advia 1200 Hematology System (Bayer, Norwood, MA).

Results

Effect of a PPAR- γ ligand on lung pathology-A hallmark symptom of allergic asthma is leukocyte infiltration in the lungs, and thickening of the cell walls lining the bronchioles. By priming mice with OVA/Alum and challenging them with IN OVA this same response is produced in the lungs (Figure 1C). PPAR- γ ligands have been shown to reduce this infiltration [6, 7]. Therefore the lungs of the mice were harvested and one lung was fixed in formaldehyde to be embedded and sectioned then stained with H&E. These lungs were analyzed for infiltration and thickening of cell walls lining the

bronchioles. The infiltration of leukocytes in lungs of mice that were primed with OVA/Alum and challenged with IN OVA show increased infiltration in the lung, and thickening of the lining of the bronchiole walls (Figure 1C). Mice that were primed and challenged this way and treated with GW1929 show significantly less infiltration in the lung and little thickening of the cell walls lining the bronchioles (Figure 1D). Mice that were not induced to develop the disease did not show these symptoms, even when treated with GW1929 (Figure 1B).

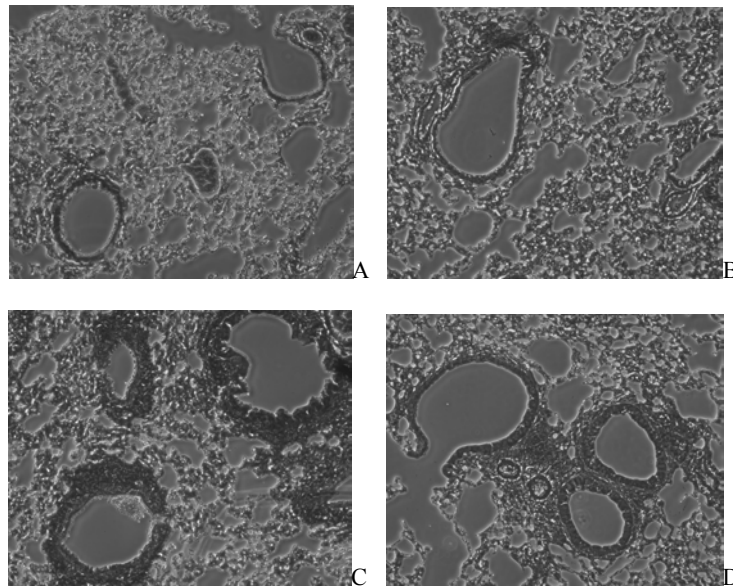


Figure 1. On day 16 mice were sacrificed and the lungs were fixed, paraffin-embedded, sectioned and stained with H&E. Balb/c mice that were primed with PBS/Alum and challenged with PBS show no infiltration or thickening of the cell wall lining the bronchioles (A). Mice primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 show increased infiltration in the lung and thickening of the epithelial walls lining the bronchioles (C). Mice that were primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 and treated with GW1929 on days 12-15 show significantly less infiltration, and little thickening of the cell walls lining the bronchioles (D). Mice that were primed with PBS/Alum and challenged with PBS and treated with GW1929 show no infiltration and no thickening of the cell walls lining the bronchioles (B).

Effect of GW1929 treatment on Airway Hyper responsiveness (AHR) – Ligands for PPAR γ have been reported to reduce inflammation [6, 7]. This suggests that PPAR γ ligands may be useful in treating allergic inflammation. We therefore used the high affinity PPAR γ ligand GW1929 in a murine model of allergic asthma to determine if PPAR γ ligands could affect the severity of symptoms in this disease. One hallmark of allergic asthma is difficulty exhaling. The Buxco whole body plethysmograph measures the animal's ability to exhale in response to exposure to the broncho-constrictor methycholine. This value is reported as PenH, a unit less number. Groups of mice were primed and challenged with OVA, a model allergen, and some groups were treated with .5 μ g GW1929 daily during intranasal exposure. Airway hyper responsiveness to exposure to methylcholine was then determined using the Buxco plethysmograph 24 hours after the final intranasal challenge. The resulting PenH values were averaged and plotted. The results show that as expected, mice that were not primed to develop allergic asthma responded to increasing concentrations of methylcholine (Figure 2). In addition,

mice primed to develop allergic asthma developed severe AHR. Treatment of mice primed to develop allergic asthma with GW1929 lead to a slight decrease in AHR. Finally treatment of mice that had not been primed resulted in normal responses to methycoline exposure.

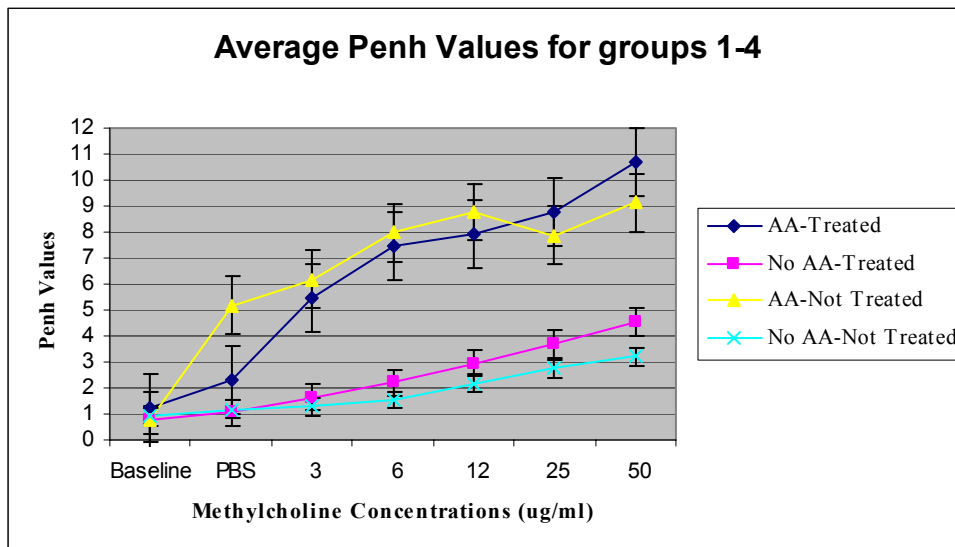


Figure 2. Mice that were primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 show a significantly higher PenH value than mice that were primed with PBS/Alum and challenged with PBS. PenH values were measured on day 16 in a Buxco Whole Body Plethysmograph. At low levels of methylcholine concentrations mice that were treated with GW1929 and primed with OVA/Alum then Challenged with IN OVA have significantly lower PenH values than mice that were not treated.

Effect of GW1929 treatment on lung Eosinophilia – Eosinophilia is a hallmark of allergic asthma. Eosinophils are attracted to the bronchial wall and lumen by an allergen IgE reaction [13]. This causes accumulation of eosinophils in the bronchial wall and lumen during allergic asthma, and is a good marker of allergic asthma [14]. The lungs of the mice from the different experimental groups were harvested and eosinophils counted using an Advia 1200 Hematology System. The averages were taken for each group and plotted below. There was a downward trend in percent eosinophils from mice that were not treated to mice that were. This suggests that less of an infiltration occurred in mice that were treated with the ligand (Figure 3).

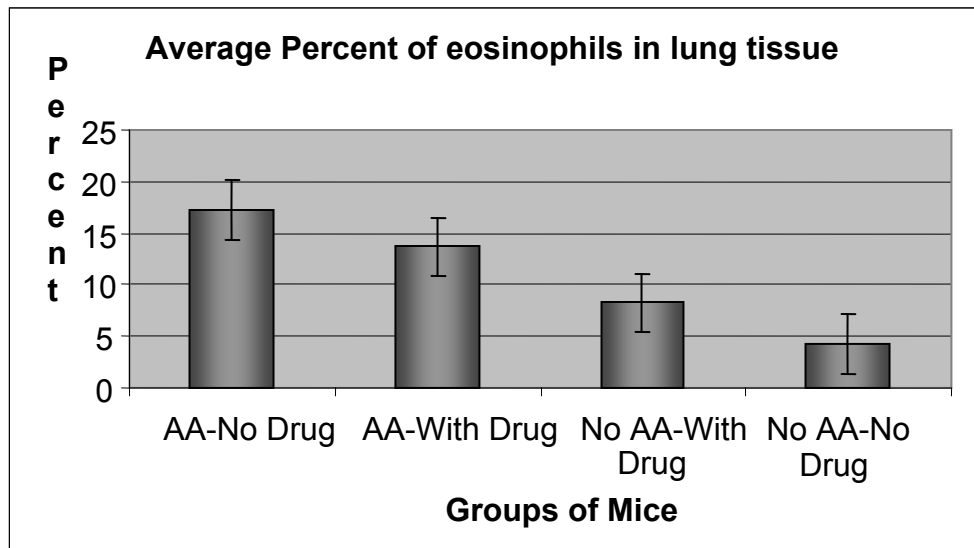


Figure 3. Mice that were primed with OVA/Alum then challenged with IN OVA have a significantly higher percent of eosinophils in the lungs than mice that were primed with PBS/Alum then challenged with PBS. The mice that were treated with GW1929, primed with OVA/Alum and challenged with IN OVA do show a downward trend in percent eosinophils in the lungs.

Conclusions

Reduced difficulty exhaling – The data above suggests that mice treated with a PPAR- γ agonist, such as GW1929, have slightly less difficulty exhaling. This implies that at least some of the symptoms of allergic asthma were relieved.

Reduced Eosinophila – Lower amounts of eosinophils in the lungs of the mice that were treated with the ligand and developed allergic asthma suggests that there was less infiltration. If there is less infiltration this would mean damage was done to the airways, therefore reducing the difficulty breathing.

Histology – The lungs of the mice that were not induced with allergic asthma clearly have no infiltration of eosinophils into the lung. Mice that were induced to develop the disease and treated with the ligand show similar lung pathology to those that were not. This suggests that the ligand helped to reduce the infiltration in the lungs.

Discussion

This experiment has many potential benefits for allergic asthma patients. This new treatment could relieve them of their allergic asthma symptoms without causing them any severe side-effects. The above data shows that GW1929 does have a therapeutic effect on allergic asthma, and therefore may hold exciting new clues to treating this disease.

We are currently conducting experiments to further analyze the effectiveness of GW1929 on relieving symptoms of allergic asthma. We are administering the treatment in different ways to determine if the symptoms are affected differently.

References

- [1] Kemp, J. P. 2002. Pediatric Asthma. Science Press; London:17-27.
- [2] Asthma Drugs and Their Known Side Effects. www.dramatic-asthma-relief.com/drugs.htm. 2000.
- [3] Powell, H., Gibson, P. 2003. Inhaled corticosteroid doses in asthma: an evidence based approach. *eMJA*. 178: 223-5.
- [4] Walsh, L.J., Wong, C.A. Osborne, J. Cooper, S. Lewis, S.A. Pringle, M. Hubbard, R. Tattersfield, A.E. 2001. Adverse effects of oral corticosteroids in relation to dose in patients with lung disease. *Thorax*. 56:279-84.
- [5] Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; et. Al. 1998. *N*-(2-Benzoylphenyl)-L-tyrosine PPAR γ agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. *J. Med. Chem.* 41:5020-5036.
- [6] Kersten, S., Desvergne, B. Whali, W. 2000. Roles of PPARs in health and disease. *Nature*. 405:421-4.
- [7] Wilson, T.M., Brown, P.J. Sternbach, D.D. Henke, B.R. 2000. The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.* 43:527-50.
- [8] Kung, T.T., Jones, H. Adams, G.K. Umland, S.P. Kreutner, W. Egan, R.W. Chapman, R.W. Watnick, A.S. 1994. Characterization of a murine model of allergic pulmonary inflammation. *Int. Arch. Allergy. Immunol.* 105:83-90.
- [9] Nakajima, H., Iwamoto, I. Tomoe, S. Matsumura, R. et.al. 1992. CD4⁺ T-lymphocytes and IL-5 mediate antigen induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.* 146:374-7.
- [10] Durham, S.R., Kay, A.B. 1985. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin. Allergy*. 15:411-18.
- [11] Mueller, C., Weaver, V. Vanden Heuvel, J.P. August, A. Cantorna, M.T. 2003. Peroxisome proliferators-activated receptor- γ . *Archives of Biochemistry and Biophysics*. 418:186-96.
- [12] Hargreave F.E., Ryan G., Thomson N.C., O'Byrne P.M., Latimer K., Juniper E.F., Dolovich J. 1981. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J. Allergy. Clin. Immunol.* 68:347.
- [13] Frigas, E., Loegering, D.A. Gleich, G.J. 1980. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab. Invest.* 42:35.
- [14] Frigas, E., Loegering, D.A. 1981. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo. Clin. Proc.* 56:345.
- [15] Na, H., Surh, Y. 2003. Peroxisome proliferators-activated receptor γ ligands as bifunctional regulators of cell proliferation. *Biomedical Pharmacology* 66:1381-91.

Biomechanical Evaluation of Crutch Design Variations

Adriana Segura, McNair Scholar, Penn State

Faculty Research Adviser

Stephen Piazza, PhD, Associate Professor

**Departments of Kinesiology, Mechanical Engineering, Bioengineering,
Orthopedics and Rehabilitation
Penn State**

Abstract

The purpose of the study is to evaluate variations in the forces and moments applied to both the axillary (armpit) pad and handgrip of standard axillary crutches. The modified axillary pad was tapered in the front rather than symmetric and the modified handgrip was angled to about 17° rather than horizontal. The crutches used could be fitted with the standard or novel versions of both the axillary pad and handgrip, permitting four different crutch configurations. Using a six-axis load cell built into one of the crutches, forces and centers of pressure were calculated at both the axillary pad and the handgrip for each configuration in ten subjects. The only significant difference found was in the location of the handgrip forces. The center of pressure on the modified handgrip was more central than the location on the standard handgrip suggesting a more uniform force distribution.

Introduction

It is important for people with disabilities to be able to comfortably use crutches. Standing and walking allow for improved growth of bone, improved circulation of blood, reduced bladder infections and reduced pressure lesions (Shortell, Kucer, Neeley, & LeBlanc, 2001). Crutch walking offers physiological and psychological advantages that a person cannot gain by sitting and using wheeled mobility. The use of crutches helps people with disabilities to be able to move around freely. However, conventional crutches can present numerous problems to the crutch user and therefore are sometimes a hindrance rather than a benefit.

Traditional axillary crutches transmit jarring forces to the wrists and shoulders and can cause injuries to the crutch user. Many crutch users suffer from a condition called crutch palsy which occurs when the outer edge of the crutch saddle damages nerves in the axilla. This can impair conduction in the damaged nerves and can lead to total or partial paralysis in some of the muscles of the arm and hand (Subramony, 1989). A patient can usually recover relatively quickly from this condition by discontinuing the use of crutches. However, there are also more serious complications resulting from prolonged axillary crutch use including formation of an aneurysm and axillary artery thrombosis (Poddar, Gitelis, Heydemann, & Piasecki, 1993). Also, conventional crutches are usually loud and crutch users sometimes feel uncomfortable with the loud noises as they are walking.

Crutches have been used for over 5,000 years and they have not changed much in that time (LeBlanc, Carlson, & Nauenberg, 1993). There have been many attempts in the last century to modify the design of the standard axillary crutch including the development of Canadian

crutches which are a combination of axillary crutches and elbow crutches; spring-loaded crutches; and rocker-bottom crutches. However, the designs have not generated much interest nor have they been successfully marketed to crutch users. It is important to develop a more effective, safe, and comfortable crutch for crutch users.

The benefits of developing a more effective crutch are not limited to a specific group of people, but spread to many different groups. Crutches are used by many people including amputees, paraplegics, people with broken bones, people with torn ligaments and many others. With a biomechanically favorable crutch, long-term crutch users as well as short-term crutch users will be greatly helped. It will provide better mobility for them without the obstacles of conventional axillary crutches. There will be less discomfort and harsh forces on the hands and axilla.

Approximately twice as much energy is required to walk with crutches than to walk without assistance (Fisher & Patterson 1981). People with disabilities often do not want to expend so much energy on a daily basis just for walking. With a less demanding and more ergonomically effective crutch, this energy gap can be lessened and more people will want to use crutches instead of wheeled mobility because the effort of ambulation will not be as high.

The purpose of this study is to develop a refined design for a crutch that has a modified axillary pad and handgrip and to determine if this new crutch distributes the axillary pad and handgrip forces more evenly.

Literature Review

Literature was reviewed for the following topics: traditional crutches; complications of crutches; and modified crutch designs.

Traditional Crutches

Crutches and other walking aids have been used for over 5,000 years and in that time, they have not changed much (LeBlanc et al., 1993). Currently, the two basic designs of crutches that are prescribed to most patients are axillary crutches and elbow crutches.

Axillary Crutches. Axillary crutches are a type of crutch that have a handgrip as well as a pad that rests against the side of the body just under the armpit. This type of crutch is used mostly by temporary crutch users (Shortell et al., 2001). Sometimes people avoid axillary crutches because of potential problems that may arise from their use such as hand, arm, and axilla problems (LeBlanc et al., 1993).

A study by Sankarankutty, Stallard, and Rose (1979) found that while subjects of their study said that ambulation with axillary crutches was less tiring than ambulation with elbow crutches, the percentage increase in heart rate from resting rate was about 20% higher for ambulation with axillary crutches compared to ambulation with elbow crutches. The researchers hypothesized that the increase in heart rate from the axillary crutches might have been due to artificial stimulation of the heart due to the contact of the top of the axillary crutch with the thoracic cage (Sankarankutty et al., 1979).

Instead of measuring energy expenditure by comparing heart rate, a study by Dounis, Rose, Wilson, and Steventon (1980) compared the amount of oxygen uptake for axillary crutch and elbow crutch ambulation. They found that oxygen uptake was less for ambulation with

axillary crutches than for ambulation with elbow crutches. They concluded that for their study, walking with axillary crutches required less energy than walking with elbow crutches. Additionally, the subjects of the study rated the use of three types of crutches according to an effort scale provided by the researchers. All of the subjects were in complete agreement that there was less effort exerted when using axillary crutches than when using elbow crutches (Dounis, Rose, Wilson, & Steventon, 1980). However, other studies have not found significant differences in energy expenditure when subjects used axillary and elbow crutches for walking. Dounis, Steventon and Wilson (1980) found no difference in energy expenditure between axillary and elbow crutches by comparing oxygen consumption. Similarly, Hall, J., Elvins, Burke, Ring, and Clarke (1991) found no differences between the heart rate of subjects when using axillary and elbow crutch designs for ambulation.

Elbow Crutches. Elbow crutches are also known as forearm crutches. Like axillary crutches, they have a handgrip, but elbow crutches only extend to the elbow. There is no bar under or near the axilla. This type of crutch is used mostly by permanent crutch users (Shortell et al., 2001). Without the bar under the axilla, there are no jarring forces there, but there are still forces at the hands and wrists. As mentioned in the previous section, there has been some evidence that elbow crutches require more energy expenditure than axillary crutches. In a perceived effort rating by subjects in a study using both types of crutches, all of the subjects said that ambulation with elbow crutches required more effort than ambulation with axillary crutches (Dounis, Rose, Wilson, & Steventon, 1980).

Complications of Crutches

Crutches have many physiological and psychological benefits to individuals who use them by allowing them to walk instead of using wheeled mobility to get around. However, even though walking with crutches has many benefits, it also has many drawbacks that sometimes hinder individuals from using them.

Body Forces. There are harsh forces on the body due to crutch walking with axillary crutches. Forces at the crutch tip are transferred directly to the hand and wrist and indirectly to the axilla (Parziale & Daniels, 1989). Wilson and Gilbert (1982) determined that the two important forces acting on the body during crutch walking are the horizontal forces on the axilla and the total load on the hands. The study found that the whole body weight is supported by the hands along with additional inertial forces. However, the axilla only has horizontal forces acting on it. In Wilson and Gilbert's (1982) study, it was determined that the peak body horizontal forces at the axilla occurred at the apex of swing-through. A force plate was used to measure the ground reaction force at the crutch tip and a force transducer system was used to measure the horizontal crutch reaction force on the axilla. According to this study by Wilson and Gilbert (1982), the crutch user's hands support 1.1 to 3.4 times his/her body weight, and the axilla support a horizontal load of about 3 to 11% of his/her body weight.

A similar study by Goh, Toh, and Bose (1986) found somewhat different results. The study found that the peak force at the hand during crutch ambulation was 44.4% of body weight which was less than found in Wilson and Gilbert's (1982) study. Also, Goh et al. (1986) tested the differences in the axillary forces when the subjects used the crutches correctly and incorrectly. When the crutches were used correctly, the axillary load was about 5% of body weight, but when the crutches were used incorrectly, the load was about 34% of body weight.

When the subjects used the crutches correctly, the posterior upper strut of the crutch was subjected to tension while the anterior strut was in compression during crutch stance phase.

While the forces on the body are greatly increased at the axilla and hands, the forces are also increased on the supporting limb during ambulation with crutches. In the study, “Lower-limb vertical ground-reaction forces during crutch walking,” Stallard, Sankarankutty, and Rose (1978) measured the ground reaction forces on the supporting limb during crutch walking with both axillary and elbow crutches. It monitored the forces when the subjects landed on one foot as well as when they landed on two feet. For all single-foot landings with both types of crutches, the average increase was 24.5% and for all both-feet landings with both types of crutches, the average increase was 35.1% as compared to landing during normal walking. A similar study by Stallard, Dounis, Major and Rose (1980) also found an increase on the supporting limb during ambulation. The study found increases in vertical ground reaction forces of about 16% as compared to normal walking. In contrast to the findings of these two studies, a study by Li, Armstrong, and Cipriani (2001), found no increase in ground reaction force on the supporting limb. However, the study did find that during partial weight bearing crutch gait, the stance phase decreased significantly on the affected limb and increased significantly on the supporting limb. The center of gravity was shifted toward the supporting limb side of the body (Li et al., 2001).

A study by Shoup, Fletcher, and Merrill (1974) consisted of a literature search and a displacement analysis of swing-through crutch gait in order to make recommendations for further crutch modifications. The researchers suggested three developments in crutch design from the results of the study. They recommended that the vertical motion of the upper body be minimized, the shock absorption at the crutch tips be minimized, and the lateral motion of the crutch tips should be minimized.

Medical Conditions. Many cases of medical complications due to crutch walking have been documented. Crutch palsy is one of the least serious and is caused by axillary crutch walking (Raikin & Froimson, 1997). This can cause patients to have lesions of the radial and ulnar nerves which can cause denervation and conduction blocks along those nerves (Subramony, 1989). Crutch palsy can also lead to partial or total paralysis of muscles innervated by the radial, median and ulnar nerves as seen in a case report by Poddar et al. (1993). In the report, electromyography found radial nerve dysfunction with denervation of the radial innervated muscles. The study by Raikin & Froimson (1997) suggests that patients can expect complete recovery once they discontinue the use of the crutches and have splinting as required.

Another condition that can be induced by crutch use is acne mechanica. Acne mechanica is when pressure, friction, or rubbing provoke acne lesions (Kang et al., 1999). Kang et al. (1999) presented a case where a long-time crutch user developed this condition.

A more serious condition is the formation of an aneurysm due to the rubbing of the axillary pad of the crutch on the axilla of the user. There have been a number of documented cases of aneurysms caused by axillary crutch use and three are presented in the case study by Feldman, Vujic, McKay, Callcott, and Uflacker (1995). In a case report presented by Thomas and Deshmukh (1973), the patient suffered from an aneurysm of the brachial artery which led to complete thrombosis. The patient had to undergo immediate surgery and a graft was used to bridge the gap in the artery.

Modified Crutch Designs

Canadian Crutches. There is a basic design of the axillary crutch, but there has also been the development of a slightly modified version called the Canadian crutch. The Canadian crutch is basically a “Cuff” crutch that integrates aspects of axillary and elbow crutches. It has a handle as well as a “cuff” that wraps around the shoulder (Stallard, Sankarankutty, & Rose, “A Comparison,” 1978). The cuff is designed to try to limit the amount of forces transmitted to the axilla during crutch walking. Since the design of the Canadian crutch is so similar to the traditional axillary crutch, it is sometimes put under the axillary crutch category (Hall J. et al., 1991).

In the study “A Comparison of Axillary, Elbow, and Canadian Crutches,” Stallard, Sankarankutty, and Rose (1978) compared the heart rates of participants when using axillary, elbow, and Canadian crutches for ambulation. The study found that the Canadian crutch either gave the lowest heart rates at speeds comparable to those on the other types of crutches, or the highest speeds at heart rates comparable to those on the other types of crutches. Overall, the Canadian crutches appeared to be less energy consuming than the axillary and elbow crutches. A related study by Sankarankutty, Stallard, and Rose (1979) found similar results. The study found that the increase of heart rate for ambulation with Canadian crutches was about 20% lower than the increase of heart rate for ambulation with elbow crutches and about 40% lower than the increase of heart rate for ambulation with axillary crutches.

A study by Dounis, Steventon, and Wilson (1980) compared the energy use of subjects using elbow crutches and Canadian crutches by using a portable oxygen meter called the Oxylog. The results suggest that Canadian crutches are more efficient than elbow crutches because Canadian crutches allow a greater walking distance per unit of energy expenditure. While this study only contained five subjects, a follow-up study with ten subjects obtained similar results (Dounis, Rose, Wilson, & Steventon, 1980). This study compared axillary, elbow, and Canadian crutches. The study strongly suggests that Canadian crutches are preferred to both axillary and elbow crutches both objectively and subjectively.

Rocker-Bottom Crutches. The idea of the rocker-bottom crutch goes back for almost 90 years. In 1918, Hall R. developed and built a modified crutch design which featured a metal rocker at the base of the crutch. He replicated the shoulder curve of the crutch as it rotates during ambulation, and applied the arc in the form of a metal rocker to the base of the crutch. While Hall, R. (1918) described some of the advantages and disadvantages of the crutch design, no experimental study was included in his paper. However, the rocker-bottom crutch did not disappear after Hall’s preliminary design. In a study by LeBlanc et al. (1993), a quantitative comparison of different axillary crutches was conducted. One of the crutches used in the study was a rocker-bottom crutch. It was essentially a modified modern version of Hall’s rolling crutch. Similar to what Hall, R. (1918) described in his paper, LeBlanc et al. (1993) found that the crutch provided a smooth gait and increased stride length. However the disadvantages of the crutch were that it was awkward because of its size on stairs and aisles, it was heavy, and it was hard to stabilize (Hall, R., 1918; LeBlanc et al., 1993).

Basford, Rhetta, and Schleusner (1990) wanted to determine differences between rocker bottom crutches and traditional axillary crutches in speed of ambulation, number of steps, heart rates and patient security. Even though the study found no significant differences between any of the above, it found that the subjects preferred the axillary crutches to the rocker-bottom crutches. A similar study by Nielson et al. (1990) found a subjective preference for the standard axillary

crutch for going up and down stairs, overall safety, and long-term use. This study also found no differences in walking performance including self-selected walking velocity, stride length, energy cost, gait efficiency, and relative exercise intensity.

Spring-loaded Crutches. The basis of spring-loaded crutches is that the extension post of standard crutches is replaced by a post with a spring mechanism in it (Pariziale & Daniels, 1989). In a study by Pariziale and Daniels, a basic design of a spring-loaded axillary crutch was compared to a standard axillary crutch. According to the findings of the study, the spring-loaded crutches reduced both the shock and maximum load at the hand and wrist when compared to traditional axillary crutches. There was a reduction of 20-25% in the stress put on the user's wrists. In a study by LeBlanc et al. (1993) that compared spring-loaded crutches to four other modified crutch designs, advantages and disadvantages were listed. The advantages were that the crutches had a lively feel, absorbed shock, and had energy return. The disadvantages found were the moving parts, the lack of rigidity, and the difficulty in ground clearance during swing-through.

Another, more recent, attempt to design a new crutch was undertaken by Shortell et al. (2001). This new elbow crutch was made of carbon fiber composite material which incorporated a spring mechanism directly into the body of the crutch. Instead of an actual spring, the researchers chose an S-curve design in which the two arcs of the S would deflect and act like a spring. Participants in the study were satisfied with the design, but felt that there was instability due to the movement in the crutch handle (Shortell et al., 2001).

Handgrip Modifications. Complications of nerve impingement and callous formations during crutch use can be attributed to the angle of the handgrip and the contour of the wooden handle (Yeakel & Margetis, 1969). The wrist naturally should be in slight ulnar deviation as opposed to radial deviation as it is during axillary crutch walking. A study by Yeakel and Margetis (1969) suggests that these problems can be eliminated with the use of poly (methyl methacrylate), a denture base repair resin. The material is putty-like so it can be molded to the hand of the specific crutch user. The study suggests that this allows the hand and wrist to be in their best structural alignment and that the handgrip distributes the body weight over the entire palm of the hand.

An article by Park, Malone, and Steglich (1952) argues for use of a tilted crutch handpiece. The researchers explain that when 35 people grasped free rod, the angle found was in a range from 5 to 30 degrees to the horizontal and that 73% of those ranged from 20-25%. Wiley (1960) also suggests that similar modifications should be made to the handgrip in axillary crutches by making it sloping to an angle of 15 degrees with the horizontal. He says that patients who have used this angled handgrip feel that it is more comfortable than when the grip is at its traditional horizontal position. Powers and Flatt (1962) suggest that further modifications should be made to the crutch handle in addition to the sloping handgrip. The researchers suggest that the diameter of the handgrip should be made larger and should be tapered near the little finger to allow for the different degrees of flexion of the digits. The increased diameter allows for a better power grip of the handle by the crutch user (Powers & Flatt).

Other Crutch Designs. Other designs of crutches exist and some studies have compared them to traditional designs of crutches. In a study by Hinton and Cullen (1982), traditional axillary crutches were compared to Ortho crutches. The Ortho crutches were made of aluminum

with single uprights instead of double uprights like those found in traditional axillary crutches. The researchers suggested that for walking over a short distance, the Ortho crutch would be less tiring for an inexperienced patient than axillary crutches.

A report done by Nova and Laura (1985) described various modifications in walking aids. One of the aids described was the IMA crutch which featured a deformable underarm support and handgrip, full contact between crutch tip and the ground for any position of the crutch, and a button that released the upper portion of the crutch. The researchers state that unlike a normal axillary pad, the pad of the IMA crutch deflects when loaded. The telescoping aspect of the crutch allows the crutch to be reduced to a shorter length when the user is sitting (Nova & Laura). However, the study provides no scientific testing of the crutch design.

Wagstaff (1984) introduced a new design for a crutch called the Dublin crutch that featured a single shaft with a protruding handgrip and slightly modified axillary pad. The study found that there was a slight significant decrease in energy expenditure when walking with the Dublin crutch than when walking with a conventional axillary crutch.

Methods

This study evaluated whether or not there were differences in the forces and points of application of the forces on the axillary pads and handgrips. Data about the forces and the moments of force on the crutch were collected in 29 Recreation Building, the Biomechanics Laboratory.

A pair of Guardian Red Dot crutches fitted for individuals between 61 and 69 inches were used in this study. Additional axillary pads and handgrips were designed and constructed in the laboratory. The modified axillary pads were made of wood and were adapted from the shape of the original pad. The front end of the pad was tapered to reduce the pressure on the front of the axilla. Both the standard and the modified axillary pads are shown in Figure 1. The modified handgrips were made of wood and were angled to 17° which allowed for increased ulnar deviation. Both the crutch handles are shown in Figure 2. The crutches were adjustable to the two types of axillary pads and the two types of handgrips. This allowed for four configurations of the crutches. The left crutch was also modified to accommodate a six-axis load cell made by Sandia National Laboratories. Only three load cell readings were used in this study: F_x (force in x-direction), F_y (force in y direction) and M_z (moment about z-axis). Figure 3 shows how the load cell was incorporated into the crutch. Figure 4 shows the whole left crutch with the load cell built into it. Figure 5 shows a sketch of the crutch with the forces and locations shown as well as the coordinate system used.

Eight healthy subjects with no known musculoskeletal problems between the ages of 19-24 were used in the study. None of the subjects in the study needed any type of assistance for ambulation. Each subject came into the Biomechanics Laboratory once and the session lasted less than one hour. All the subjects signed Informed Consent forms prior to their participation in the study. Each subject was given instructions on how to walk with the crutches using a swing-through gait pattern. The subjects were explained that most of their body weight should be on the hands and not the axilla during swing-through.

For each of the four crutch configurations, each subject was given a five minute practice session or as long as needed to feel comfortable with the crutches around the laboratory. After the practice session, the subject was given a two minute rest period or as long as needed to feel

adequately rested. The subject then performed three good trials with each crutch configuration by walking over the force plate with a swing-through crutch gait. A trial was considered good if the left crutch tip struck the force plate and the subject adequately cleared the force plate.

The force plate used was a Kistler Company force plate. The Motion Analysis System used was the Eagle System made by Motion Analysis Corporation.

Figure 1: Standard (left) and modified (right) axillary pads



Figure 2: Standard (top) and modified (bottom) handgrips



Figure 3: Load cell incorporated into left crutch



Figure 4: Complete left crutch with load cell built into it

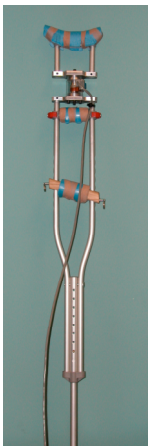
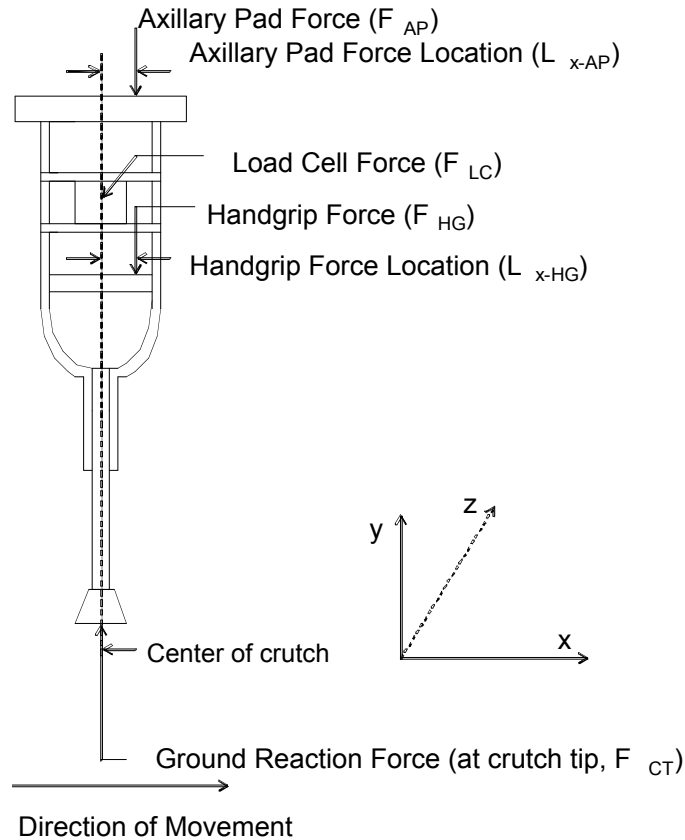


Figure 5: Sketch of crutch with forces and locations



From the forces and moments at the load cell (F_x , F_y , M_z) and the ground reaction forces when the crutch was at its vertical position, the forces and locations of the resultant forces at the handgrip and axillary pad were calculated using the equation $F = ma$. Figure 4 shows the forces and the locations on the crutch. The location is given in mm from the center of the crutch. All locations found were positive referring to the front half of the crutch. The forces and locations were found by putting the equations into a program in MATLAB. The program was run once for each subject and the forces and locations at the handgrip and axillary pad were calculated.

The three equations used for the forces at the handgrip were:

- 1) $F_{HGx} + F_{LCx} + F_{CTx} = 0$
- 2) $F_{HGy} + F_{LCy} + F_{CTy} = 0$
- 3) $M_{LCz} + M_{CTz} + L_{x-HG} F_{HGy} + L_{y-HG} F_{HGx} = 0$

Assuming:

$$a = 0$$

$$F_{HGz} = 0$$

$$M_{HGz} = 0$$

The three equations used for the forces at the axillary pad were:

- 1) $F_{APx} + F_{LCx} = 0$
- 2) $F_{APy} + F_{LCy} = 0$
- 3) $M_{LCz} + L_{x-AP} F_{APy} + L_{y-AP} F_{APx} = 0$

Assuming:

$$a = 0$$

$$F_{APz} = 0$$

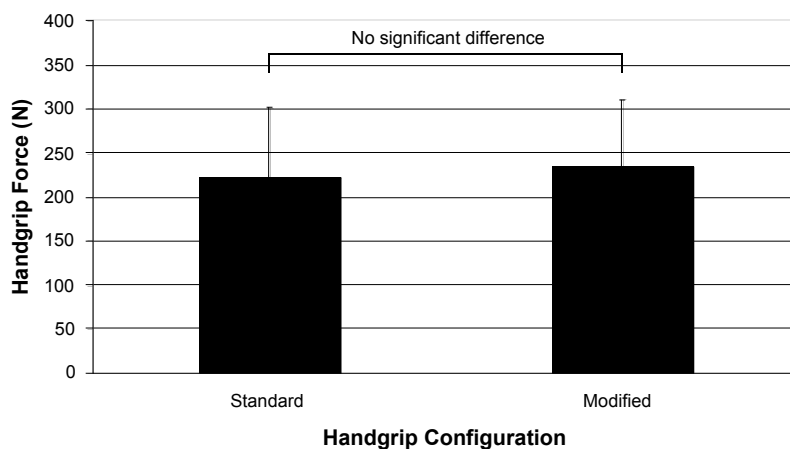
$$M_{APz} = 0$$

A two-way Analysis of Variance (ANOVA) with repeated measures was used to analyze the effects of handgrip (standard and modified) and axillary pad (standard and modified). A p-value of less than 0.05 was considered significant. The factors were the axillary pad and the handgrip. The levels for each were standard and modified.

Results

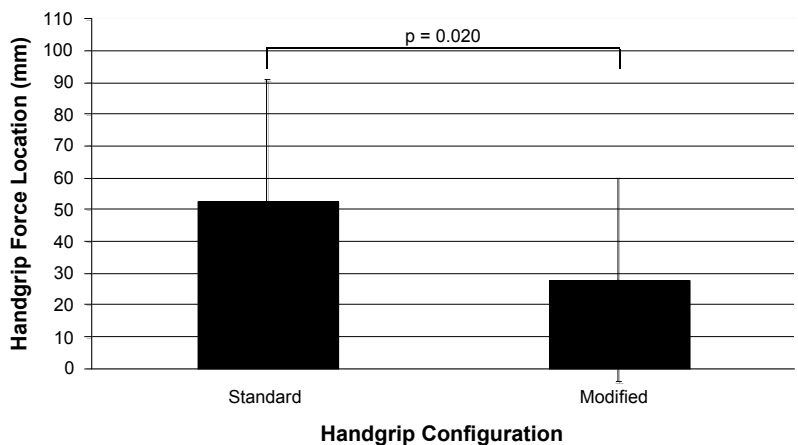
The only significant difference found was in the locations of the force for the handgrip designs. The forces on the handgrip and the forces and locations on the axillary pad were not significant.

Figure 6: Forces on the standard and modified handgrips



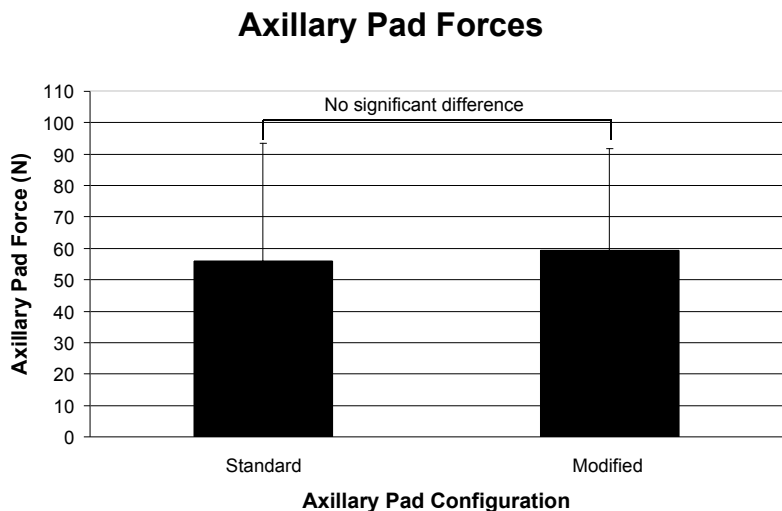
There was no significant difference found in the forces on the standard and modified handgrip designs ($p = 0.471$).

Figure 7: Force Locations for the standard and modified handgrips



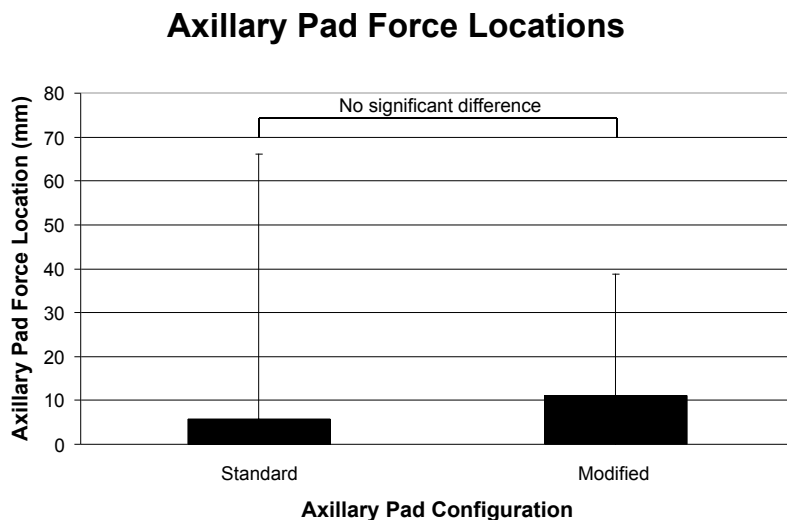
There was a significant difference found in the force location on the standard and modified handgrip designs ($p = 0.020$). The average standard handgrip force location was 52.9mm from the center of the crutch. The average modified handgrip force location was 27.9mm from the center of the crutch.

Figure 8: Forces on the standard and modified axillary pads



There was no significant difference found in the forces on the standard and modified axillary pad designs ($p= 0.434$).

Figure 9: Force Locations for the standard and modified handgrips



There was no significant difference found in the force location on the standard and modified axillary pad designs ($p= 0.699$).

The subjects were asked to rate the comfort level of each of the crutch configurations. Five of the eight subjects preferred both the standard handgrip and axillary pad. The main reason cited for preferring the standard handgrip over the modified handgrip was that the modified handgrip was larger and harder to grip.

Discussion

The point of application of the resultant handgrip force is closer to the center of the handgrip for the modified design than for the standard design. Since the force location is closer to the center of the crutch, this suggests that the body weight is distributed more evenly on the hand as predicted. For the standard design, the resultant force is applied much more to the front of the hand. This suggests that the forces are not distributed evenly in this configuration. For long-term crutch users, using this modified design for the handgrip may help to lower the jarring forces on the hand by the crutch handle. The angled handgrip design can potentially provide more comfort to crutch users by distributing the forces along the hand instead of concentrating the forces on only the front of the hand.

Five of the eight subjects preferred both the standard handgrip and axillary pad designs. However, since the main reason given for preferring the standard handgrip was that it was smaller and easier to hold, the angled shape of the modified handgrip may not have been taken much into account. The study aimed to compare the shapes of the two handgrips, but the comfort to the subjects may have relied more heavily on the size rather than the shape.

The sample size of eight people that was used for the study was very limited and was not able to be used to make generalizations for all crutch users. The subjects who participated in the

study are all from the State College, PA area and therefore are not representative of the whole population. The demographics of State College may not be similar to the demographics of the whole population. If more subjects had been used, then perhaps more significant difference may have been detected in the forces at the handgrip and forces and locations at the axillary pad. However, the differences found were so slight that hundreds of subjects may have been needed to detect any real significant difference.

Additionally, the subjects that were used were healthy college-age people. The data for the subjects may not be representative of actual crutch users because the subjects in the study did not have any problems with ambulation. The swing-through portion of the gait cycle of the subjects will be considerably different from the swing-through portion of many crutch users because many crutch users wear immobilizing leg casts. Those crutch users need extra room for the straightened leg to clear the ground. Two of the participants were experienced crutch users so their ambulation with the crutches was likely more efficient than the rest of the participants who did not have as much practice with crutches. Some of the data may not have been representative of what would have been found by using experienced or long-term crutch users.

The leading cause of non-traumatic lower-leg amputation is diabetes mellitus (Mathur & Shiel, 2004). According to Mathur & Shiel (2004), recent information estimates that 13 million people in the United States have diabetes. Many diabetics are overweight or obese and therefore crutch dynamics for those individuals may be different than the crutch dynamics of healthy subjects. None of the subjects used in the study were obese and therefore they may not have tested these crutch biomechanics.

In future studies, the modified designs for both the standard and modified handgrips and axillary pads should be made from the same material and should be the same size when comparing the shapes alone. Also, using only experienced crutch users or giving inexperienced crutch users more practice time would help to make sure that all the subjects were walking as efficiently as possible. The energy expenditure of the subjects using each of the crutch configurations was not measured in this study. However, the extra energy required for crutch ambulation is a large hindrance to many crutch users. It is one of the main reasons why many disabled people choose wheeled mobility instead of using crutches. Comparing the energy expenditure between the four combinations would be another good indication of the efficiency of the designs. Also, further modifications besides simply changing the handgrip and axillary pads would be good to explore. For example, adding a spring to the bottom of the crutch in hopes to absorb some of the shock might also produce more significant results in the future. Also, testing different handgrip and axillary pad shapes than those used in this study would also be helpful.

While this study only found significance in the location of the handgrip forces, that information shows promise in reducing the jarring pressure at the hand. The information found in this study provides groundwork for future studies in the area of crutch dynamics.

References

- Basford, J.R., Rhetta, M.L., & Schleusner, M.P. (1990). Clinical Evaluation of the Rocker Bottom Crutch. *Orthopedics*, 13 (4), 457-450.
- Dounis, E., Rose, G.K., Wilson, R.S.E., & Steventon, R.D. (1980). A Comparison of Efficiency of Three Types of Crutches Using Oxygen Consumption. *Rheumatology and Rehabilitation*, 19, 252-255.
- Dounis, E., Steventon, R.D., & Wilson, R.S.E. (1980). The use of portable oxygen consumption meter (Oxylog) for assessing the efficiency of crutch walking. *Journal of Medical Engineering and Technology*, 4(6), 296-298.
- Feldman, D.R., Vujic, I., McKay, D., Callcott, F., & Uflacker, R. (1995). Crutch-Induced Axillary Artery Injury. *Cardiovasc Intervent Radiol*, 18, 295-299.
- Fisher, S.V., & Patterson, R.P. (1981). Energy Cost of Ambulation with Crutches. *Archives of Physical Medicine & Rehabilitation*, 62, 250-256.
- Goh, J.C.H., Toh, S.L., & Bose K. Biomechanical study on axillary crutches during single-leg swing-through gait. *Prosthetics and Orthotics International*, 10, 89-95.
- Hall, J., Elvins, D.M., Burke, S.J., Ring, E.F. J., & Clarke, A.K. (1991). Heart rate evaluation of axillary and elbow crutches. *Journal of Medical Engineering and Technology*, 15(6), 232-238.
- Hall, R. (1918). A Rolling Crutch. *J.A.M.A.*, 70, 666-668
- Hinton, C.A., Cullen, K.E. (1982). Energy Expenditure During Ambulation with Ortho Crutches and Axillary Crutches. *Physical Therapy*, 62(6), 813-819.
- Kang, Y.C., Choi, E.H., Hwang, S.m., Lee, W.S., Lee, S.H., & Ahn, S.K. (1999). Acne Mechanica Due to an Orthopedic Crutch. *Cutis*, 64, 97-98.
- LeBlanc, M.A., Carlson, L.E., & Nauenberg, T. (1993). A Quantitative Comparison of Four Experimental Axillary Crutches. *Journal of Prosthetics and Orthotics*, 5(1), 20-28.
- Li, S., Armstrong, C.W., & Cipriani, D. (2001). Three-Point Gait Crutch Walking: Variability in Ground Reaction Force During Weight Bearing. *Archives of Physical Medicine & Rehabilitation*, 82, 86-92.
- Mathur, R., & Shiel, W.C. Jr. (June 2004). *Diabetes – David Meets Goliath*. Retrieved June 15, 2004, from <http://www.medicinenet.com/script/main/art.asp?articlekey=17281>

- Nielsen, D.H., Harris, J.M., Minton, Y.M., Motley, N.S., Rowley, J.L., & Wadsworth, C.T. (1990). Energy Cost, Exercise Intensity, and Gait Efficiency of Standard Versus Rocker-Bottom Axillary Crutch Walking. *Physical Therapy*, 70(8), 487-493.
- Nova, L.C., & Laura, P.A.A. Design and Use of Improved Walking Aids. (1985). *Journal of Biomedical Engineering*, 7, 329-333.
- Pariziale, J., & Daniels J. (1989). The mechanical performance of ambulation using spring-loaded axillary crutches. *American Journal of Physical Medicine and Rehabilitation*, 68(4),193-195.
- Park, H.W., Malone, E.W., & Steglich, R. (1952). The Tilted Crutch Handpiece. *Archives of Physical Medicine*, 731-733.
- Poddar, S.B., Gitelis, S., Heydemann, P.T., & Piasecki, P. (1993). Bilateral predominant radial nerve crutch palsy. *Clinical Orthopaedics and Related Research. A Case Report*, 297, 245-6.
- Powers, W.R., & Flatt A.E. (1962). Modified Crutch Handle. *Archives of Physical Medicine & Rehabilitation*, 43, 570-573.
- Raikin, S., & Froimson, M. (1997). Bilateral Brachial Plexus Compressive Neuropathy (Crutch Palsy). *Journal of Orthopedic Trauma*, 11(2), 136-137.
- Sankarankutty, M., Stallard, J., & Rose, G.K. (1979). The Relative Efficiency of 'Swing Through' Gait on Axillary, Elbow and Canadian Crutches Compared to Normal Walking. *Journal of Biomedical Engineering*, 1(1), 55-57.
- Shortell, D., Kucer, J., Neeley, W.L., & LeBlanc, M. (2001). The design of a compliant composite crutch. *Journal of Rehabilitation Research and Development*, 38(1), 23-32.
- Shoup, T.E., Fletcher, L.S., & Merrill, B.R. (1974). Biomechanics of Crutch Locomotion. *Journal of Biomechanics*, 7, 11-19.
- Stallard, J., Dounis E., Major, R.E., & Rose, G.K. (1980). One Leg Swing Through Gait Using Two Crutches. *Acta orthop. Scand.*, 51, 71-77.
- Stallard, J., Sankarankutty, M., & Rose, G.K. (1978). A Comparison of Axillary, Elbow, and Canadian Crutches. *Rheumatology and Rehabilitation*, 17, 237-239.
- Stallard, J., Sankarankutty, M., & Rose, G.K. (1978). Lower-limb vertical ground-reaction forces during crutch walking. *Journal of Medical Engineering and Technology*, 2(4), 201-202.
- Subramony, S.H. (1989). Electrophysiological findings in crutch palsy. *Electromyogr. clin. Neurophysiol.*, 29(5), 281-285.

- Thomas, J.M., & Deshmukh, N. (1973). Aneurysm of the Brachial Artery with Complete Thrombosis Caused by a Crutch. *The American Surgeon*, 389-399.
- Wagstaff, P.S. (1984). The Energetics of Walking using Axillary Crutches and the Prototype of a New Design Termed the Dublin Crutch. *Physiotherapy*, 70(11), 422-424.
- Wiley, B.C. (1960). Crutch with Sloping Handgrip. *J.A.M.A.*, 172(7), 694-695.
- Wilson, J.F., & Gilbert, J.A. (1982). Dynamic Body Forces on Axillary Crutch Walkers During Swing-Through Gait. *American Journal of Physical Medicine*, 61(2), 85-92.
- Yeakel, M.H., & Margetis, P.M. (1969). A Customized Crutch Hand Grip. *The American Journal of Occupational Therapy*, 23(5), 410-412.