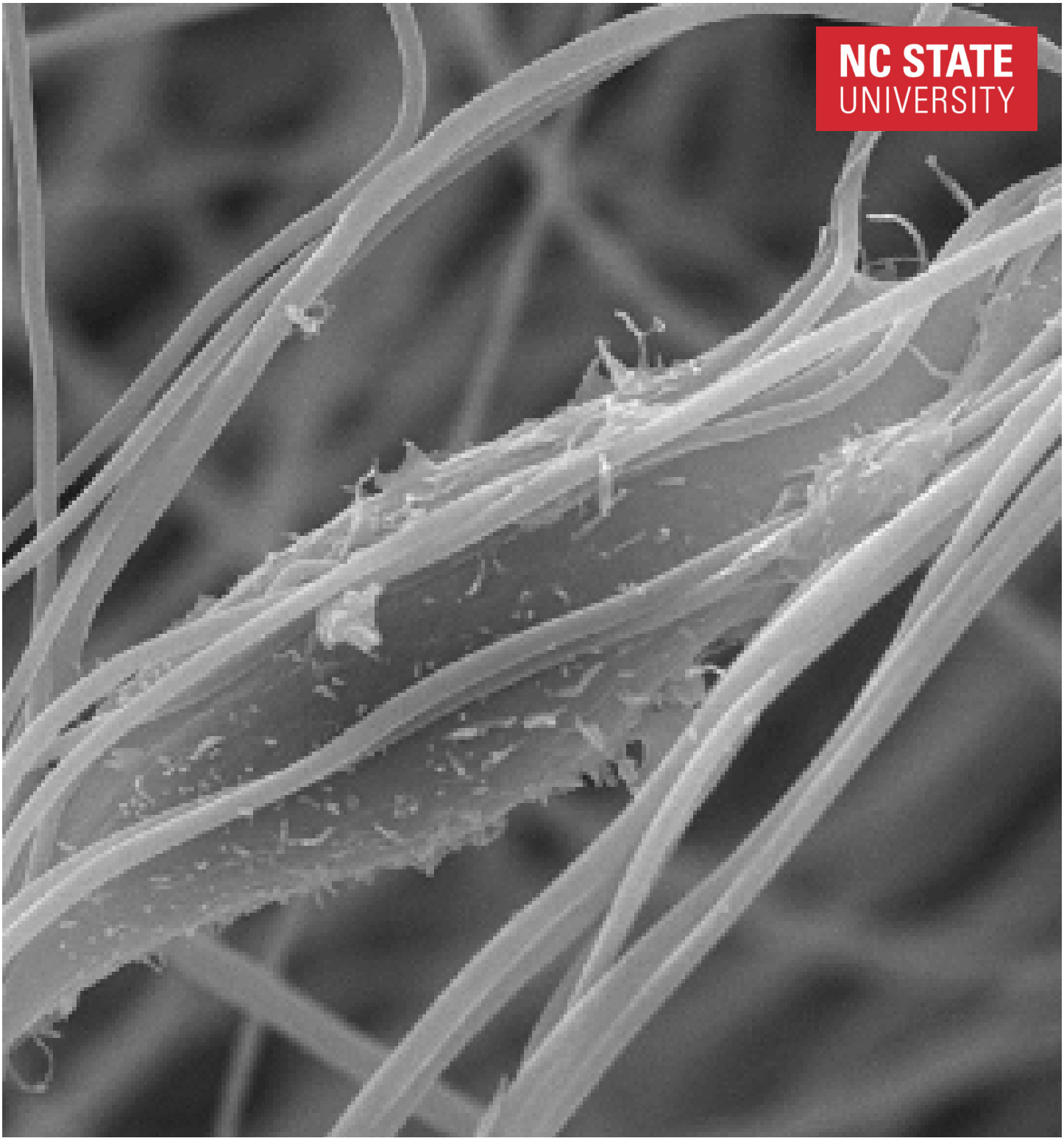


INK

The Undergraduate Research Journal at NC State University

Spring 2017

INK
Spring 2017

Cover image was provided courtesy of the undergraduate research of **Matthew Hartman** at NC State from "Murine Fibroblast Response to Aligned Polylactic Acid Nanofibers" beginning on page 22 of this issue. The image is of a relatively small fibroblast growing along a set of parallel fibers taken at 2500x.

EDITORIAL BOARD

EDITOR'S NOTE

It is with great pleasure that we present the 2017 Spring Issue of *INK: The Undergraduate Research Journal at NC State University*. This long-awaited issue comes five years after the last issue of *INK* was published in 2012. The Editorial Board has worked tirelessly to revitalize the journal and ensure its future as a continued presence at NC State.

This issue exhibits some of the best work of undergraduate students at NC State. Articles range from independent studies of innovative textile fibers, urban planning, and engineering, to animal biology and scientific reviews. At NC State, we "Think and Do." *INK* showcases students who put the essence of this motto into action.

The Editorial Board would like to acknowledge the mentorship of *INK*'s faculty advisor, Dr. Christopher Ashwell, Director of the Office of Undergraduate Research (OUR), in addition to Ms. Judy Day and Ms. Heather King also from OUR. We would also like to thank the peer reviewers who came from multiple disciplines at NC State to dedicate much time and energy to reviewing articles. The Editorial Board would additionally like to express gratitude for the guidance provided by Mr. William Cross, Ms. Lillian Rigling, and Mr. Darby Orcutt, from NC State Libraries. Finally, we would like to thank the student authors and mentors of the articles featured in this issue who demonstrated excellence within both the technicalities and communication aspects of their research.

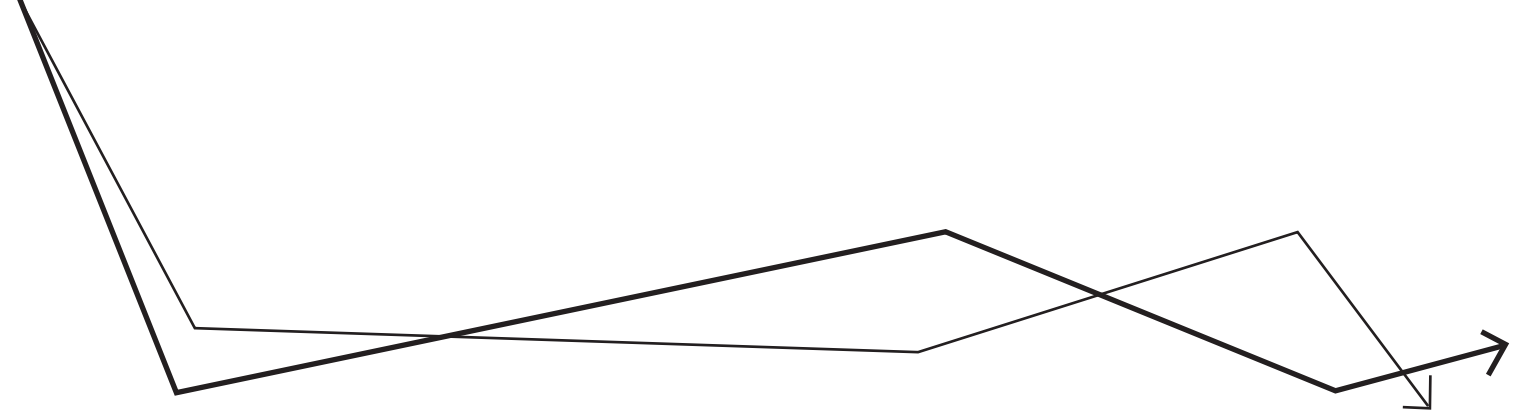
We are excited for you to engage in the inspiring research of NC State's undergraduate students. It is our hope that Dr. George Barthalmus, late Director of the Office of Undergraduate Research who initiated the journal in 2004, would be proud of this endeavor and the continuation of a journal he so passionately supported. Our Editorial Board looks forward to working toward the expansion of undergraduate research and the sharing of research through publication. *INK* is dedicated to continuing to embody NC State's "Think and Do" tradition within the future of undergraduate research.

Gratefully,

Ashle M. Page
David Nacouzi
Editors-in-Chief

TABLE OF CONTENTS

REVIEW ARTICLES



IDENTIFICATION OF KEY DRIVERS FOR MUNICIPAL UTILITY PERFORMANCE

by **Jasmin Alsaied, Mohamed Bourham, Ph.D. & Michael Fusco, Ph.D.**

Jasmin Alsaied is a senior in nuclear engineering with a minor in mathematics and naval science at NC State University. She would like to thank Andy Fusco for his continued support in her research endeavors. Jasmin will be commissioning into the U.S. Navy as a Surface Warfare Officer with the USS Lassen.

Mohamed Bourham, Ph.D. is the Alumni Distinguished Graduate Professor of Nuclear Engineering, and Director of College of Engineering Master of Engineering Program at NC State University. His interests include plasma-matter interaction, plasma propulsion and thrusters, fusion engineering, materials synthesis and coatings, nuclear and mixed waste disposal, drycasks and high-level waste packaging studies. He works in the areas of experimental and computational high heat flux irradiation of materials, studies on multi-layered coatings and corrosion barriers for high level waste packages, and research on evaluation of materials' corrosion using electrochemical techniques of cyclic potentiodynamic polarization.

Michael Fusco, Ph.D. presented a thesis on the investigation of the corrosion of protective coatings on steel for high-level waste canisters. He currently works as a postdoctoral research associate at the Department of Chemical and Biomolecular Engineering at NC State University.

> Abstract

This report explores the various performance indicators for municipal electric utilities and the greatest impact financial investments can make for improving these indicators. A literature search provided key detail about performing an analysis that would prove useful to utilities to hone in on performance indicators that allowed for the most prudent of investments. Data mining techniques and statistical analyses were performed on data sets concerning the 51 North Carolina municipal electric utilities to identify several key ratios and performance indicators that have the greatest impact on cost of service, system reliability, and customer satisfaction. Statistical analyses were used to determine which investments optimized these outcomes. No other studies on the statistical significance of municipal electric utility performance indicators were identified in our literature search.

Keywords: municipal utility performance, cost analysis of service, electric reliability.

> Introduction

Performance indicator data provides a useful measure of the municipality's financial health over a period of time. A study using statistical modeling to identify performance indicators for municipal electric utilities to make prudent financial investments has never been performed as described in this paper. Regardless of size or customer demand, each municipality aims to meet its revenue requirements, decrease its operating expenditures per fiscal year, and maintain a high level of service to its customers, among other things. However, conclusions to this analysis suggest that certain variables are more important to a municipality's operating expenditures, margins, and overall financial health, than other performance indicators. This report examines the relationships between the variables that have the greatest impact on key areas of municipal electric utility performance.

>Literature Review

In De, Bandyopadhyay, and Chakraborty's (2011)[1] paper in the Journal of Business Studies Quarterly, a factor analysis on financial ratios was performed based on the results of a cluster analysis of the Indian cement industry. Initially, 44 variables were grouped into seven categories and selected as possible financial ratios of interest for the cement industry. After the exclusion of low inter-correlation variables, the study conducted a factor analysis, identifying eight key variables to the Indian cement industry. The paper is useful in comparison to our analysis for many reasons. The analytical process described in De, et. al. is useful because of the selection of homogeneous companies (or municipalities, in our case) for analysis. Additionally, the study recognizes the various aspects of the financial position of each company. This aspect is crucial in understanding and analyzing the municipalities, as they each vary vastly in size, demand, and revenue requirements. Lastly, the study outlines its procedure in determining which financial ratios it deems prudent to the cement industry's investments, which is most important for our study.

In *Municipal Benchmarks: Assessing Local Performance and Establishing Community Standards* by David N. Ammons[2], the author discusses specific benchmarks that have been collected by prime examples of municipal-run electric services. Said performance targets aided our process in defining variables that were key drivers as well as deciding which performance factors to look at in the first place.

Lastly, *Applied Multivariate Statistical Analysis*[3] by Richard Johnson and Dean Wichern aided our search for accurate analyses to run with our specific set of data, and what to render the results as when processed. This resource provided our study with methods for determining our correlation coefficients and methods of identifying outliers.

> Objectives of the Study

The objective of this study is to aid municipal electric utilities in either confirming or modifying their financial investments to improve their cost of service, reliability and customer satisfaction using the analysis techniques described herein. These techniques allowed us to select only a few key ratios and performance indicators that made the most impact on the investments of a utility. It is necessary to understand the local economic considerations, financial health, and demographics of each municipality to better understand what kind of prudent investments it will benefit most from.

> Theoretical Analysis

To provide numerical confirmation of specific variables that may be key indicators of financial health, spreadsheet analysis techniques were used to analyze data found from all sources. Municipal electric utility members of ElectriCities of North Carolina, Inc. are required to respond to data requests of governmental organizations and the North Carolina Department of State Treasurer to prepare state-mandated and regulated financial documents each year to remain transparent to constituents and stakeholders of their financial health. These documents, called Comprehensive Annual Financial Reports (CAFRs), provided line items detailing the spending, revenues, margins, and overall financial health of the municipal's various city-run services, like electric services, water, gas, cable, sewage, etc. This study used the electric fund information, usually found in the enterprise portion of each city's CAFR, to conduct its spreadsheet analyses.

Fiscal year 2013 CAFRs were the source of over 80% of the municipal data. If a city had combined water and power funds, or an enterprise fund that could not be separated from the electric fund, the municipality was left out of the study. Several iterations of this study were performed to narrow down the list of performance indicators and key

ratios that significantly impacted the optimization of capital investments. Municipalities from both North Carolina Municipal Power Agency Number 1 (PA1) and North Carolina Eastern Municipal Power Agency (EA) that provided sufficient financial detail in their respective CAFRs were used for the initial analyses. Added to these 51 cities were non-member North Carolina cities, such as Fayetteville, New River Light and Power, and Kings Mountain, that had accurate data for that fiscal year concerning profits and expenditures. Variables were calculated using the guidelines set by the American Public Power Association (APPA) ratio report[4].

Another source of data that was used in this study were electric utility performance indicators. ElectriCities collects performance indicator data, which are calculated ratios, detailing the overall financial health, margins, and reliability of the utility. These ratios, calculated using utility-submitted data over the course of a year, serve as a benchmark against other municipal utilities' reliability performance and investment. In conducting this portion of the analysis, several out-of-state municipalities were included if they possessed accurate and complete data, because the number of in-state reporting municipal electric utilities was limited. The out-of-state data set came from APPA[5].

Our goal in accumulating these data through data mining was to provide a statistically significant pool of data for our analyses. In our data mining attempt, usable data were identified for all 51 North Carolina member cities serviced by ElectriCities. However, as the pool was widened to non-participants and out-of-state municipal electric utilities, the data became more sparse and unusable. For example, several performance indicators were calculated differently in the state of California, while some municipalities combined their electricity and water services, not allowing for us to separate their profits for our use. Some statistical analyses were deemed unusable or insufficient due to the smaller pool of data, thus requiring us to attempt widen the pool with accurate data. For example, ratios using miles of line were essentially not included for non-member cities due to data unavailability. Because of the limitations of the data caused by differentiating reporting methods, lack of reporting, and sheer unavailability, the reliability of our pool of data was limited to the accurate data provided by the 51 member cities in North Carolina[6]

We hoped to analyze the performance of customer service, system reliability, cost of service, safety, and overall financial health. However, due to a lack of available data, only cost of service, system reliability, and financial health could be studied. From the variables that are studied, customer service was deemed to be the most difficult to analyze. Smaller municipalities generally do not measure customer service, and there was no numerical measure of customer service at municipalities that was readily available for this study. After selecting variables, procuring data for these variables proved to be a challenge. Not every municipality reported data, nor did they report data using the same parameters, so missing and or flawed data was frequently encountered during the data mining procedure. As stated earlier, only accurate and complete data were used in the data pool, thus forcing our study to discard any inaccurate or more likely, incomplete or vague data.

> Variable Selection

Variables were selected from reliability ratios, performance indicators, and financial health indicators all compiled by ElectriCities of North Carolina. Additionally, several variables were added from the APPA ratio report, to help account for ratios that are less likely to be calculated by the municipality in the CAFRs. By combining all these variables into one spreadsheet, a correlation matrix was constructed to determine which variables had the highest correlation with municipal performance. The following table was used to determine the validity of a variable[7].

Table 1 - A summary of possible correlation coefficients, as defined by *Applied Multivariate Statistical Analysis.*

Possible Correlation Coefficients	
1	Perfect positive relationship
0	No relationship
0<x<1	Indicates positive correlation
-1>x>0	Indicates negative correlation
-1	Perfect negative (inverse) relationship
x>|.5|	Variable chosen as a strong key factor
x<|.5|	Variable thrown out as a weak key factor

After choosing several variables from the initial correlation matrix, which only used data from member cities of ElectriCities of North Carolina, several other analyses were run to further identify key factors that could drive municipal utility investments. Variables with the highest correlation were graphed. To identify nonlinear relationships, independent variables were graphed normally, squared, to the 0.5

power, and as a natural log. From these graphs, the dependent variables were graphed regularly, and each graph contained a linear and exponential trendline. However, this graphing exercise suggested that that all relationships were primarily linear. Additionally, different performance indicators were compiled by developing normalized ratios. This new set of indicators tracked things like spending habits of municipalities per kWh sold and the OSHA incidence rate of municipalities and were added to further investigate variables that may enhance municipality performance. Using ratios in this manner helped to normalize the indicators over a range of sizes for municipal electric systems.

A regression analysis was performed, where the multiple R was analyzed to determine the significance of the correlation between the independent and dependent variable in each case. Correlations setting the normalized ratios against each other were run to go through the possibility of all variables, providing some insight into the variables that may most affect municipality performance. Additional correlation matrices were compiled using these variables to ensure that no additional variables were missed. Each of the graphed variables was put under a regression analysis. In this analysis, we were interested in the correlation of Net Income per Distribution Cost and Net Income per Power Supply Cost versus O&M (operations and maintenance) per Retail Customer. The absolute value of the natural log and square root of both Net Income per Distribution Cost and Net Income per Power Supply Cost were plotted against O&M per Retail Customer. Distribution Cost is calculated as expenditures less power supply cost. Total revenue less power supply cost was thrown out due to data limitations. The most interesting graph format of the variables was the natural log of the independent variable versus the dependent variable, as it was an excellent way to see where cities stand in terms of financial health. Municipalities that were closer to where ln(x) approaches infinity are effectively maximizing their return and net profit. However, those municipalities closer to where ln(x) approaches 0 should investigate their spending and investments.

> Data Analysis

The first step in determining variables for financial health and investments was to accurately capture and calculate all of the possible variables that would be used in the analysis. Below is a summary of each variable included in the study[8]:

1. *Residential Revenue/kilo-watt hour:* Revenue from all residential customers per kWh sold
2. *Commercial Revenue/kWh:* Revenue from all commercial customers per kWh sold
3. *Industrial Revenue/kWh:* Revenue from all industrial customers per kWh sold
4. *Total Revenue/kWh:* Revenue from all customers per kWh sold
5. *Debt to Total Assets:* Ratio of long-term debt, plus current and accrued liabilities, to total assets and debits
6. *Operating Ratio:* Ratio of electric operation and maintenance expenses to total electric operating revenues
7. *Current Ratio:* Ratio of total current and accrued assets to total current and accrued liabilities
8. *Net Income per Revenue Dollar:* Ratio of net electric utility income to total electric operating revenue
9. *Operating and Maintenance Expense per kWh sold (O&M/ kWh sold):* Electricity utility operation and maintenance expenses, including cost of generated/ purchased power, to total kWh sold (resale/ultimate)
10. *Purchased Power Cost per kWh*: Ratio of total costs of power supply to total kWh purchased
11. *Distribution Costs of O&M per Retail Customer:* Total distribution operation expenses to customers
12. *Distribution Costs of O&M per Circuit Mile:* Ratio of total distribution operation expenses to total number of circuit miles of distribution line
13. *Administrative and General Expenses per Retail Customer:* Ratio of total electric utility administrative and general expenses to the total number of retail customers
14. *OSHA Incidence Rate:* Lost workday cases during year to total worker-hours of exposure/100 employees
15. *Energy Loss Percentage:* Ratio of total energy losses to total sources of energy
16. *System Load Factor:* Average load, total sales plus losses divided by 8760 hours, to system peak demand

Acquiring consistent and useable data proved to be a challenge due to limitations with the data sources. Since CAFRs vary from city to city, it was necessary to calculate A&G (administrative and general costs) and O&M costs outside of the CAFRs, rather than find them as detailed line items on the budget. For example, a city may have had a line item for truck repairs, but another city may not have. Additionally, outliers found in the variable graphs and data mining efforts provided exceedingly high correlation between variables that were least likely to be correlated with one another. However, when outliers were removed, correlations returned to their expected normal coefficients, and correlations disappeared. For example, one electric worker in Benson fell and was injured for an extensive period, making Benson's OSHA incidence rate much greater than it normally would be. Due to this isolated incident, OSHA incidence rate initially had a greater relationship with other variables, but after further examination, it was determined that the variable was no longer significant with the exclusion of outliers.

Secondly, reliability indices were calculated. These indices were included in the study with the intent of helping municipal utilities identify areas in which to invest in their distribution system to improve reliability. Due to the unavailability of reliability data with smaller municipalities served by ElectriCities, out-of-state municipalities were included to obtain a statistically significant sample size. Three reliability indices were used, shown below:

SAIDI (System Average Interruption Duration Index): average outage duration for each customer served, measured in units of time (hours, minutes)

$$SAIDI = \frac{sum\ of\ all\ customer\ interruption\ durations}{total\ number\ of\ customers\ served}$$

SAIFI (System Average Interruption Frequency Index): average number of interruptions that a customer would experience

$$SAIFI = \frac{total\ number\ of\ customer\ interruptions}{total\ number\ of\ customers\ served}$$

CAIDI (Customer Average Interruption Duration Index): average outage duration that any given customer would experience; can also be average restoration time of power

$$CAIDI = \frac{sum\ of\ all\ customer\ interruption\ durations}{total\ number\ of\ customer\ interruptions}$$

In studying the various municipal electric systems, overall size played a role in the level of technology deployed in each system. It was determined that there must be some way to identify the quality, or rating, of the city's system. To address this issue, a municipality's technology level was devised, which was based on the availability of three technologies for a municipality. A level 1 municipality is characteristic of one with 200-2,500 customers with a considerable amount of outage minutes. However, a level 4 municipality usually has over 20,000 customers, and frequent outages, but with short durations. SCADA, or Supervisory Control and Data Acquisition, allows utilities to monitor systems remotely. Some SCADA systems allow the opportunity to open/close breakers and control other devices on their distribution system. GIS, or Geographical Information System, maps the utility equipment and uses a database similar to Google Maps to display the equipment. Mapped equipment includes poles, transformers, meters, etc. GIS serves as an asset management tool, considerably reducing field work necessary due to the viewing capabilities on the mapped display. AMI, or Advanced Metering Infrastructure, allows meters to be read remotely using a communication network between the municipal electric utility and meter, avoiding the need to send an electric worker to a site to read or disconnect a meter. Lastly, OMS, or Outage Management System, utilizes AMI and notifies the utility if a customer has lost power, avoiding the need for a customer to call in an outage. Advanced AMI systems can also lo ElectriCities nearest to the outage and predict where the outage stems based on the locations of other customers without power. A detailed technology requirement per level is provided in Table 2.

Table 2 - A summary of the available technologies per level, as determined by ElectriCities of North Carolina.

Technology Level	Description
Level 1	No SCADA
Level 2	SCADA only
Level 3	SCADA and GIS
Level 4	SCADA, GIS, OMS (or AMI only)

The technology level helped differentiate the municipalities, but did not entirely solve the issue of missing data. However, the data indicated that smaller cities seemed to only be able to maintain distribution, and had minimal funds to make improvements. The issue of improving a system with continual outages is one they encounter and are aware

of, but they simply cannot provide capital for the investment.

After identifying the possible variables to use in the study, the next step of the process was to construct correlation matrices in order to identify potential relationships between the variables. Using the correlation matrix, it was identified that

$$(System)\ Load\ Factor\ vs.\ \frac{O\&M}{kWh\ sold}$$

and

$$\frac{Debt}{Total\ Assets}\ vs.\ \frac{Purchased\ Power\ Cost}{kWh}$$

had strong correlation coefficients. While relationships between other variables in Figure 1 had higher correlations, such relationships appeared to be intuitively obvious and lacked value in terms of providing a utility with useful information about investments or actions that could improve operating results. Figures 2 and 3 show the respective graphs for the analysis of the independent variables identified in the correlation matrix provided in Figure 1. These variables were plotted using square root and natural log of the dependent variable. Outliers were identified and discarded from further data analysis if the data behind the outliers was questionable. Additionally, regression analyses were performed on the variable pars, and the multiple R was calculated using Excel regression statistics. The largest multiple R calculated was

$$\ln(Load\ Factor)\ vs.\ \frac{O\&M}{kWh\ sold}$$

Load Factor appears to be one of the variables that has the greatest impact on operational costs. Tables 3 and 4 show the regression statistics of each variable analyzed.

The multiple R was calculated using Excel regression statistics. The largest multiple R calculated was for

$$\sqrt{\frac{Debt}{Total\ Assets}}\ vs.\ \frac{Purchased\ Power\ Cost}{kWh}$$

and this appears to be a linear relationship. We can also conclude from this analysis that Debt to Total Assets is one of the variables that has the greatest impact on operational costs.

A second correlation matrix was compiled using additional variables. The highest non-trivial correlations identified in the second correlation matrix were

$$\sqrt{\frac{Net\ Income}{Power\ Supply\ Cost}}\ vs.\ \frac{O\&M}{customer},\ \sqrt{\frac{Net\ Income}{Distribution\ Cost}}\ vs.\ \frac{O\&M}{customer},$$

$$\ln\left(\frac{Net\ Income}{Power\ Supply\ Cost}\right)\ vs.\ \frac{O\&M}{customer},$$

and $\ln\left(\frac{Net\ Income}{Distribution\ Cost}\right)\ vs.\ \frac{O\&M}{customer}$.

Figures 4 and 5 provide scatter plots for these relationships across the dataset. As stated earlier, each graph was fitted with an exponential and linear trendline, to help identify outliers and nonlinear relationships once determined to have a high correlation coefficient using the first correlation matrix. In cases where a municipality was identified as an outlier, the municipality was discarded from the data pool if the underlying quality of data for that municipality was deemed to be inconsistent or in question. From these graphs, we can assume that a reasonably strong linear relationship exists between the net income per power supply cost (a modified ratio made using APPA calculations) and O&M per customer as well as a strong correlation of these two variables with municipality performance.

Using the relationships displayed in Figures 4 and 5, a regression was run to determine the largest R^2 value. From the above graphs, the largest R^2 value corresponds with the graph of

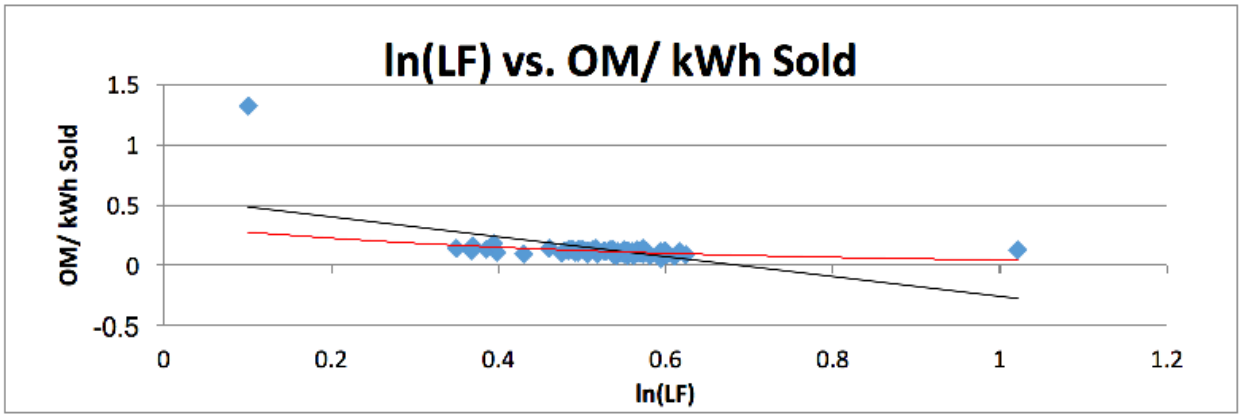
$$\sqrt{\frac{Net\ Income}{Distribution\ Cost}}\ \text{and}\ \ln\left(\frac{Net\ Income}{Power\ Supply\ Cost}\right).$$

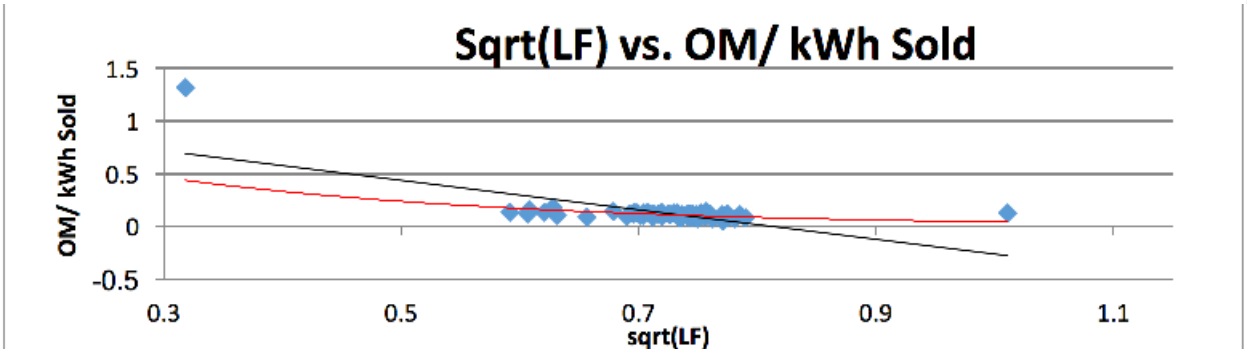
Regressions were then run and the multiple R was calculated using Excel regression statistics. The largest multiple R calculated was for

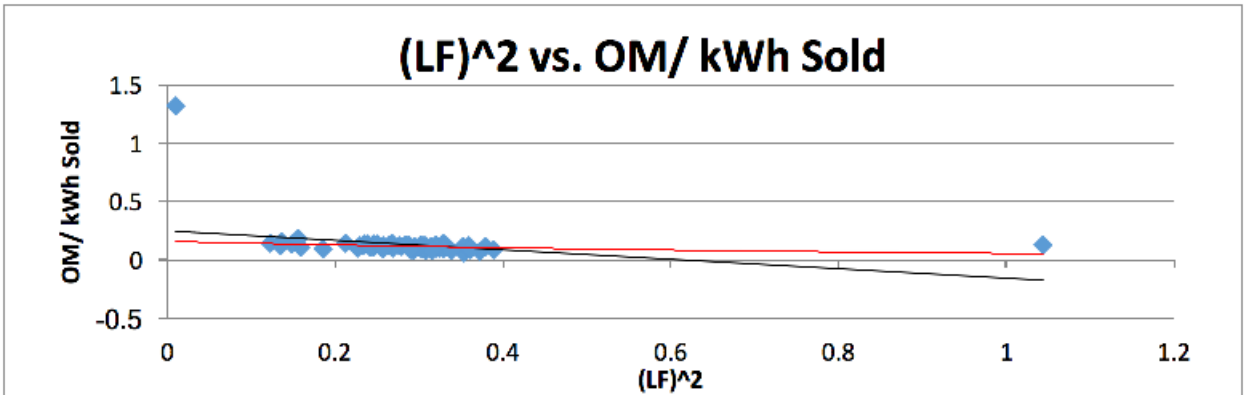
$$\sqrt{\frac{Net\ Income}{Distribution\ Cost}}\ \text{vs.}\ \frac{O\&M}{customer}.$$

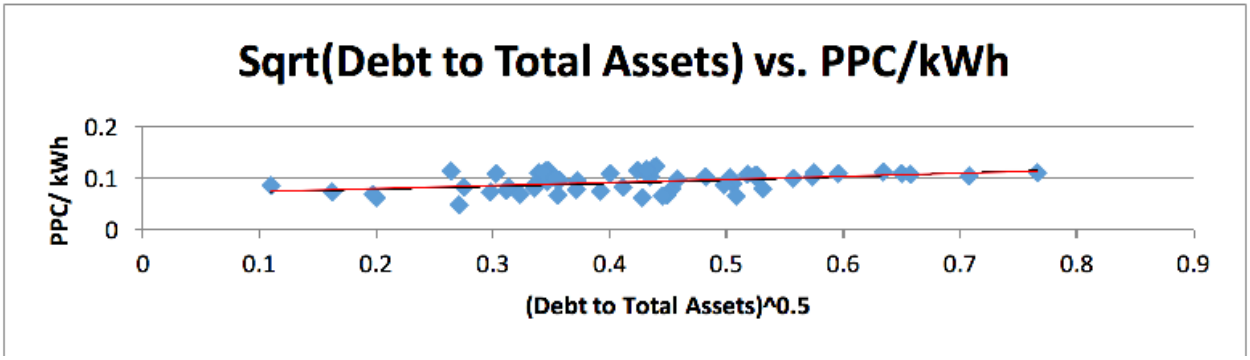
We can conclude from this relationship that $\frac{O\&M}{customer}$ is an indicator of the effectiveness of a municipal utility investments and can heavily impact investment strategy. Table 5 shows the regression statistics of each variable analyzed.

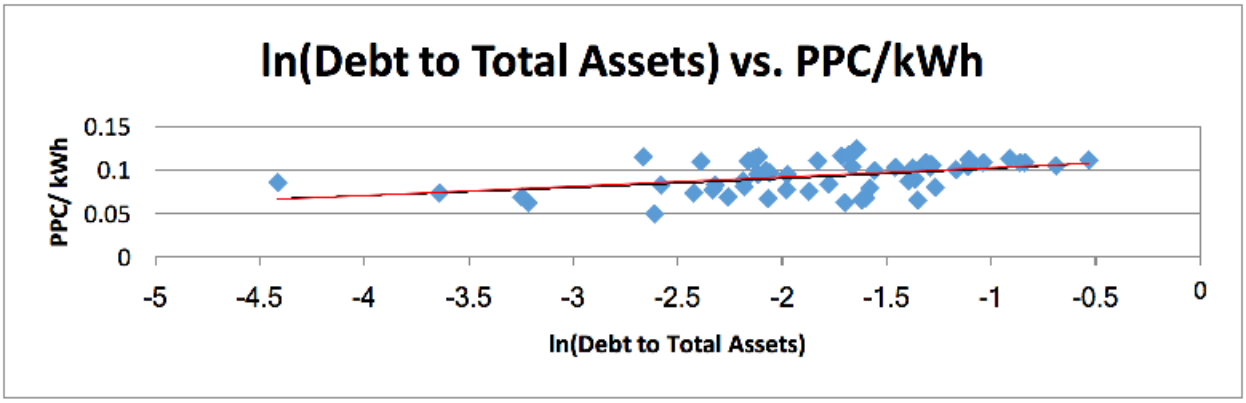
A third correlation matrix was compiled using the reliability and technology level data. Though some reporting differences were encountered, data were found from the APPA website, which included out-of-state municipalities to widen the pool due to a larger availability of data than before. Figure 6 shows the third correlation matrix derived from data retrieved from the APPA online reports. With municipalities that had higher technology level scores, they had overall better reliability time and less outage time per customer per outage. However, correlations between financial metrics and the reliability indices were relatively low. Therefore, additional analysis on the reliability indices was not

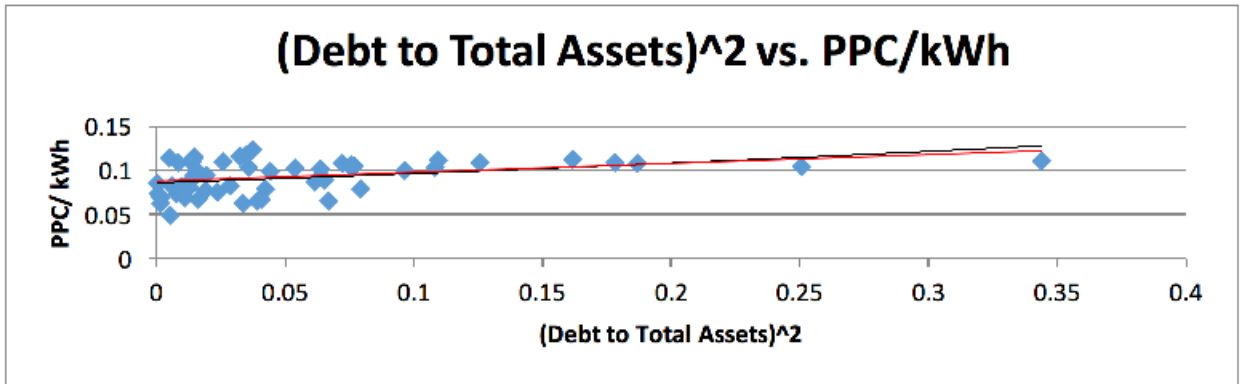

ln(LF) vs. OM/ kWh Sold
OM/ kWh Sold
1.5
1
0.5
0
-0.5
0
0.2
0.4
0.6
0.8
1
1.2
ln(LF)


Sqrt(LF) vs. OM/ kWh Sold
OM/ kWh Sold
1.5
1
0.5
0
-0.5
0.3
0.5
0.7
0.9
1.1
sqrt(LF)


(LF)^2 vs. OM/ kWh Sold
OM/ kWh Sold
1.5
1
0.5
0
-0.5
0
0.2
0.4
0.6
0.8
1
1.2
(LF)^2


Sqrt(Debt to Total Assets) vs. PPC/kWh
PPC/ kWh
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
(Debt to Total Assets)^0.5


ln(Debt to Total Assets) vs. PPC/kWh
PPC/ kWh
0.15
0.1
0.05
0
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
ln(Debt to Total Assets)



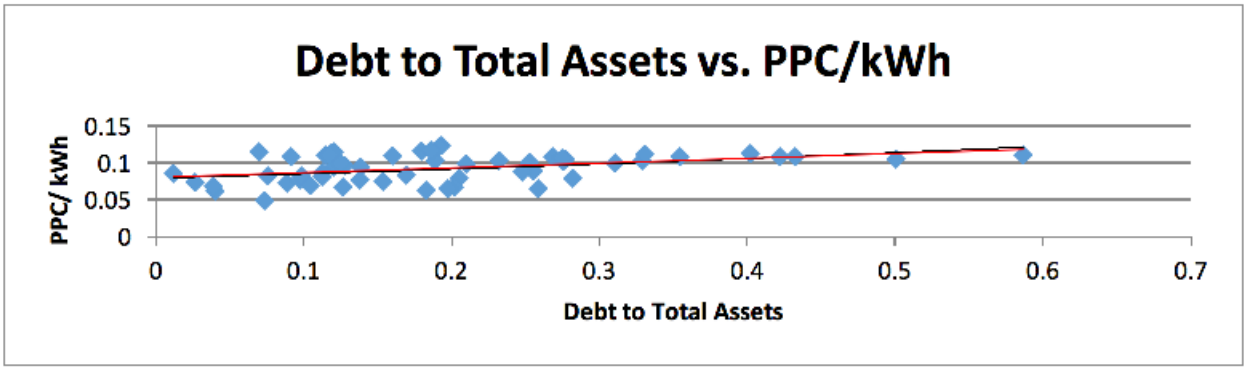


Figure 3 - Graphs of the independent variable, debt to total assets manipulated for non-linear relationships as well as graphs of the independent variable, system load factor, manipulated for non-linear relationships.

Table 3 - A summary of the regression statistics from the System Load Factor vs. O&M/kWh sold

Regression of SLF vs. OM/ kWh sold	
Regression Statistics	
Multiple R	0.5540
R Square	0.3069
Adjusted R Square	0.2940
Standard Error	0.1368
Observations	56
Regression of (SLF)^2 vs. OM/ kWh sold	
Regression Statistics	
Multiple R	0.3183
R Square	0.1013
Adjusted R Square	0.0847
Standard Error	0.1558
Observations	56
Regression of (SLF)^0.5 vs. OM/ kWh sold	
Regression Statistics	
Multiple R	0.6968
R Square	0.4855
Adjusted R Square	0.4760
Standard Error	0.1179
Observations	56
Regression of ln(SLF) vs. OM/ kWh sold	
Regression Statistics	
Multiple R	0.8269
R Square	0.6837
Adjusted R Square	0.6779
Standard Error	0.0924
Observations	56

Table 4 - A summary of the regression statistics from the Purchased Power Cost/ kWh sold vs. Debt to Total Assets.

Regression of PPC vs. D2A	
Regression Statistics	
Multiple R	0.4061
R Square	0.1649
Adjusted R Square	0.1495
Standard Error	0.0171
Observations	56
Regression of PPC vs. (D2A)^2	
Regression Statistics	
Multiple R	0.3542
R Square	0.1255
Adjusted R Square	0.1093
Standard Error	0.0175
Observations	56
Regression of PPC vs. sqrt(D2A)	
Regression Statistics	
Multiple R	0.42299
R Square	0.17892
Adjusted R Square	0.16371
Standard Error	0.01695
Observations	56
Regression of PPC vs. ln(D2A)	
Regression Statistics	
Multiple R	0.41362
R Square	0.17108
Adjusted R Square	0.15573
Standard Error	0.01703
Observations	56

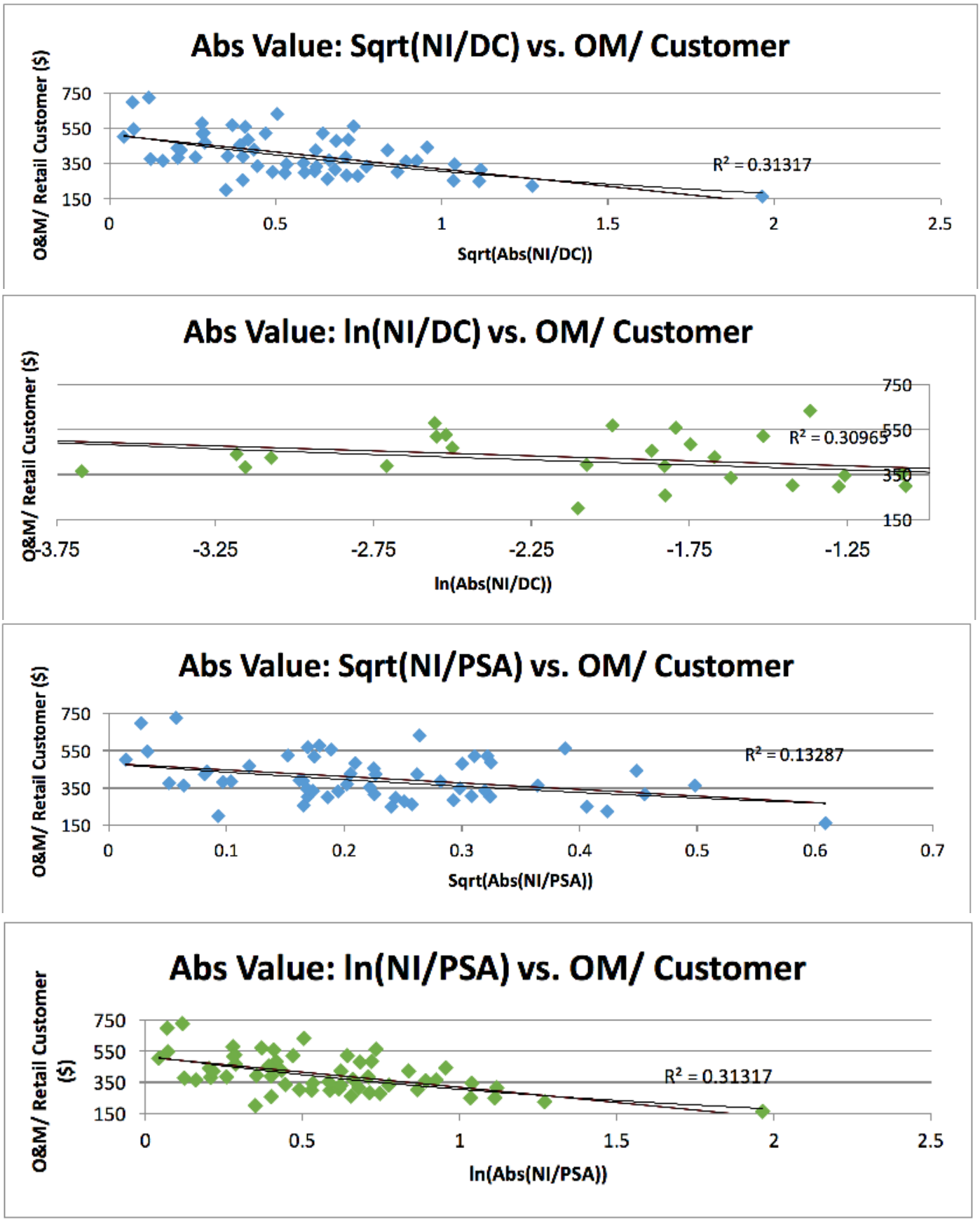


Figure 5 - Graphs of the independent variable, net income per power supply cost, manipulated for non-linear relationships.

Table 5 - A summary of the regression statistics from the Net Income/Distribution Cost vs. O&M/Customer and Net Income/ Power Supply Cost analysis.

Regression of NI/ DC vs. O & M/ Retail Customer	
Regression Statistics	
Multiple R	0.442
R Square	0.196
Adjusted R Square	0.181
Standard Error	110.338
Observations	56
Regression of (abs(NI/ DC))^0.5 vs. O & M/ Retail Customer	
Regression Statistics	
Multiple R	0.5596
R Square	0.3132
Adjusted R Square	0.3005
Standard Error	101.9521
Observations	56
Regression of ln(abs(NI/ DC)) vs. O & M/ Retail Customer	
Regression Statistics	
Multiple R	0.5565
R Square	0.3097
Adjusted R Square	0.2969
Standard Error	102.2128
Observations	56
Regression of (abs(NI/PSA))^0.5 vs. O&M/ Retail Customer	
Regression Statistics	
Multiple R	0.3645
R Square	0.1329
Adjusted R Square	0.1168
Standard Error	114.5552
Observations	56
Regression of ln(abs(NI/PSA)) vs. O&M/ Retail Customer	
Regression Statistics	
Multiple R	0.3917
R Square	0.1534
Adjusted R Square	0.1378
Standard Error	113.1879
Observations	56

	Debt To Total Assets	*Operating Ratio*	*Current Ratio*	*Net Income/ Revenue Dollar*	*O&M/Retail Customer*	*A&G/# Customers*	*SAIDI (w/o MED)*	*SAIFI (w/o MED)*
Debt To Total Assets	1.000							
Operating Ratio	-0.127	1.000						
Current Ratio	-0.386	-0.191	1.000					
Net Income/ Revenue Dollar	0.125	-1.000	0.191	1.000				
O&M/Retail Customer	0.420	-0.117	-0.265	0.119	1.000			
A&G/# Customers	-0.066	0.257	-0.126	-0.252	-0.044	1.000		
SAIDI (w/o MED)	0.327	-0.065	-0.166	0.061	0.072	-0.246	1.000	
SAIFI (w/o MED)	0.208	-0.049	-0.235	0.051	-0.049	-0.145	0.606	1.000

Figure 6 - Correlation matrix of selected variables, using reliability data from APPA.

warranted. For future work, it is recommended that the reliability indices be correlated against a wider range of data (if available) to see if there is a reasonable correlation between a financial indicator and a reliability index.

Figures 3 - 6 and Tables 3 - 5 are shown below:

> Conclusion

The largest issue in identifying meaningful variables was the issue of data relevance, availability, and techniques of calculation. For example, each municipality may have many of the performance indicators that are mandated to be reported. However, due to the difference in customer class, size, and system structure, you must question whether or not the parallels you are drawing and the analyses you are running are accurate. Additionally, some information is simply not available. Smaller municipalities struggle to place investments towards bettering their distribution systems, and can simply not be expected to track certain reliability factors as well as display line items of their distribution expenditures on their CAFRs. This situation made the issue of identifying reliable data difficult, and forced us to widen our pool of municipalities to ensure that the data pool was large enough to provide us with accurate correlations.

The study provided herein provides a basis for the discussion of providing data for studies of this nature in the future. Our study found it difficult to locate all the data necessary for the statistical analyses initially planned for this research. In the future, institutions like the APPA and other municipal electric utility organizations could collect accurate data from all municipalities regardless of size or demand concerning reliability (all calculated the same way) as well as general financial health ratios not available in the CAFR, such as those specifically calculated for this report. In addition, developing a common rating system for customers to use concerning their experience with customer service representatives, outage crew personnel, etc. would be helpful in reintroducing this variable into this study. A rating system focused on customer service with numerical ratings on friendliness, timeliness, and issues resolution would aid municipalities in seeing if adequate training is being provided for representatives and to indicate whether or not customers are satisfied with the service they are receiving. Those experiencing high numbers of outages may note that in their survey, thus giving the municipality an idea of an area of line or equipment that may need replacement. This will help future studies of this nature to investigate different variables that may improve municipality financial health, cost of service, reliability and customer service.

The variables that provided the best relationships on the financial health of the municipals were

$$\frac{Net\ Income}{Distribution\ Cost}$$

and System Load Factor. By providing several methods of statistical analysis to confirm this, we were able to provide an outline for city governments to follow in the future to better track their capital expenditures and investments.

It is important to ensure that customer satisfaction, reliability, and cost of service is up to par with what customers are expecting. The variables that have been isolated through the statistical methods described above form an approach in measuring the reliability of your municipal electric system and cost of service per number of customers, or circuit mile. An APPA-led or independent survey effort to further gain data concerning municipalities of all sizes and customer demand classes would be effective for municipalities to compare themselves to similar cities to gain a better understanding of where prudent investments can be made. In the future, further studies concerning prudent investments for municipalities can help bring smaller cities into more technologically developed and financially stable distribution systems and can help maintain larger distribution systems' customers and demand.

> Endnotes

1. De, A., Bandyopadhyay, G., Chakraborty, B., Paruya, S., Kar, S., & Roy, S. (n.d.). Application of Factor Analysis on the Financial Ratios of Indian Cement Industry and Validation of the Results by Cluster Analysis. *Journal of Business Studies Quarterly,2*(3), 13-31. Retrieved February 20, 2015, from http://jbsq.org/wp-content/uploads/2011/06/June-2011-B.pdf

2. Ammons, D. *Municipal benchmarks: Assessing local performance and establishing community standards*. Sage Publications; Thousand Oaks, California; 2012.

3. Johnson, R., & Wichern, D. *Applied multivariate statistical analysis*. Prentice-Hall; Englewood Cliffs, NJ; 2007.

4. American Public Power Association. *2013-2014 Annual Directory & Statistical Report*. Washington, DC; 2014.

5. American Public Power Association. *2013-2014 Annual Directory & Statistical Report*. Washington, DC; 2014.

CONTEMPORARY ARTISTS IN FLORENCE

by **Monica Galletto**
Mentor: **Russell Flinchum, Ph.D.**

Monica Galletto currently works as a designer at Red Hat, Inc.'s global headquarters in Raleigh, NC. Monica graduated summa cum laude from NC State University in Spring 2016 with a Bachelor in Graphic Design and a minor in Business Administration. During her undergraduate career she was involved in the University Honors Program and held positions as an Honors Fellow, Resident Advisor, Design Editor for Windhover, and Design Ambassador for the College of Design. In her field of study she is interested in designing impactful user experiences for individuals and sharing the narratives of local communities through design.

> Abstract

There is a tendency for people to accept art that they are familiar with and to value the old over the new. Florence, Italy, serves as a prime example of this phenomenon due to the fact that millions of tourists visit each year to view the city's historic artworks; however, this city also hosts a community of contemporary artists, which is often overshadowed by the famous works of deceased artists. Contemporary Artists in Florence (CAF) seeks to bring awareness to a portion of those contemporary artists by sharing their works and personal journeys of creating new art in a historic environment. These stories are documented through interviews, photography, and film, and are compiled into a print magazine and video series. The project educates artists and art enthusiasts about the influence that the past has over present makers and how people assign artistic value. Ultimately, this study reveals the struggles these artists face, shares their creative contributions, and teaches how we as the public can grow our appreciation for living makers.

> Introduction

The Italian Renaissance, centered in Florence, produced many revolutionary artists whose creations are still acclaimed around the world. Even in our contemporary society there is a tendency for people to accept art that they are familiar with and to value the old over the new. Why is it that people are so attracted to historic art and deceased artists? This initial question inspired Contemporary Artists in Florence (CAF).

Generally, there is a lack of critical study and analysis regarding contemporary makers and their techniques in the realms of art and design. In the education system, there is a profound emphasis on historic creators, but that same rigor of study is rarely applied to their living counterparts. Florence serves as an optimal location to begin such an analysis since there is a rich contrast between its historical and contemporary producers.

The classical vocabulary that was revived during the Italian Renaissance was founded in perfection, idealism, and realistic detail. CAF develops a commentary on the following questions: Given that art of historic importance holds more value than the methods and subject matter used in contemporary works, how does this influence modern day makers? If the past triumphs over the present in the realms of art and design, then how do contemporary creators stand up to the competition of renowned artists whose works are stationed in famous museums? This project shifts the spotlight from the Italian Renaissance, which the public is already so familiar with, and reveals the creative contributions local makers in Florence are developing for visual creators everywhere. The project explores how modern-day makers create new work in a city focused on the past; it also reveals their techniques and inspirations. CAF also aims to provide the participating artists with recognition, which they often do not receive in their own city.

CAF serves as a resource that documents the creative advances in Florence that are being made today. Completing this study in Florence also creates a narrative on how people assign value to creative works. The investigation produced in this research also delves into the complexities of meaning and originality surrounding contemporary art and will help future creators to critically assess the value they assign in their own work.

> Research Methodology

After the initial research, drafting of proposals, generation of consent forms, and approval from North Carolina's Institutional Review Board for the Use of Human Subjects in Research, a task list for the project scope was created. This included methods to invite artists and designers to participate in the study. These individuals were invited via online community groups, such as the Creative People in Florence Facebook group, and in person through studio visits and related events, such as the Florence Design Week. Following this stage was a four-step interview process carried out with each of the participants.

The First Encounter

After inviting artists and designers to the study, the researcher met with potential participants to discuss the scope of the project and visit the individual's studio. If there was a fit, both parties signed a consent form and additional dates were established for the following interviews. In total, 14 creatives agreed to participate: three jewelry artists, three sculptors, two painters, one architect, one graphic designer, one visual artist, one photographer, one etcher, and one graphite artist.

Touchstone Tour

A Touchstone Tour is a design research method that is used as a means of generating conversation and discussion. It is meant to provide context, and it involves interacting with artifacts in a specific environment (Martin and Hanington, 2012, p. 184). The tours are meant to immerse the researcher in the participant's world, which is why the tours for this study took place in the studio of the participant. During the tour, both parties were able to interact with the artwork, which provided a foundation of understanding to the artist's particular story, behavior, and personality.

The Interview

The interviews were semi-structured where the researcher drew from the same list of questions for every participant. While the questions were pre-written, participants were interviewed in a conversational manner so that the artists could truly recount their story, unedited. Occasionally, based on their responses or from trends that appeared during the Touchstone Tour, the researcher had the freedom to generate new questions on the spot. The structured questions covered four categories that inquired about the participants' personal background, their

artistic style, their experience in regards to working in Florence, and how they perceive artistic value. These interviews were filmed, recorded, and later transcribed by the researcher.

Observation and Review

The final encounter consisted of the researcher presenting the transcribed interview to the participant for review and final approval. Interviews were slightly shortened to include information pertinent to the study or to exclude information that was unnecessarily personal. Participants were able to review the original interview in full and see which parts were selected to be shown in the final study. The transcriptions were also edited for grammar and clarity's sake, and all edits were made clear. The participants had full freedom to change any details of the interviews during this meeting. This encounter also consisted of a creative demonstration on behalf of the artist with the researcher filming. This gave closure to the study as it connected their responses and story with their work.

Further Work

After the interviews, the researcher isolated trends and patterns noted from the participants' interviews. The interviews, along with photography, were produced into a print magazine, video footage was produced into individual videos for the participant's interviews, and the collection of work gathered was presented online.

Interview Videos:
https://www.youtube.com/
watch?v=EdChijAHUFo&list=PLCNE2aZ9SnjIF
R3TC3usKnOP96xEQ1uTc

Online site: http://contemporaryartistsinflorence.
wordpress.com

> Results

The responses of the artists carried some similar trends which were used to answer the initial questions of this research study. Their responses supported the following conclusions: Choosing to be an artist is a difficult decision for individuals; the contemporary arts in Florence are not given sufficient attention; The city of Florence tends to influence the work of its living artists; it is easier for artists in Florence to show their work outside of Florence and Italy; Florence is standoffish to new or different art; understanding historic art is necessary to understanding and appreciating contemporary art; there are benefits to having an international community in Florence.

These themes are mostly characterized by the fact that the artists are creating their work in Florence. There were pros, like having a unique, international community with constant inspiration and stimulation, but there were also cons, like difficulty showing work in Florence, being surrounded by people with a narrow view of what art should look like, and not having enough support from the local government. Note that in a few cases some responses actually opposed the majority.

The difficulties of being an artist in Florence were evident from the sentiments shared among the artists. It was clear that the government mirrored the tendencies of the public, which was to put the focus and funds on the historic works. It is apparent that the city could provide more support to its living creative since this is currently lacking. Creative People in Florence, a grassroots group of artists, is one example of a group in the city, but at the time of the research it was not a governmental source of support. The Strozzina is one example of an institutional organization in Florence that brings in contemporary work; however, their exhibitions do not always feature Florentine makers. Museo Novecento, provides a rich look into the contemporary arts of 20th century Italy, and some of its history focuses on Florence in particular; yet, not all the individuals they feature are currently living or creating work. Prato, a city just outside Florence, serves as a modest example of a city that caters to 21st century Italian artists. Alongside its historic attractions, one can also find a large community of contemporary artists at the grand Centro per l'Arte Contemporanea Luigi Pecci.

The interviews also generated data to answer questions concerning how people generate value. After gathering the artists' responses about why they personally create art and how people tend to assign value to art, it became clear that these two reasons converged:

Art is valuable when . . .	My purpose for creating is . . .
. . . someone can connect with it	. . . for people to connect with it
. . . it is made authentically	. . . to inspire others and leave a legacy
. . . the work is human/alive	. . . to understand life
. . . it makes people think about something/it tells a story	. . . to explain or communicate something

> Further Discussion

During the research, additional trends outside the scope of the project's intended reach were noted. These included the following: female artists experience more hardships than men, there is a divide between those artists whose profession is either supported or not supported by their families, there is a growing expat community in Florence, Italy, and due to the historic nature and reputation of Florence, artists and designers feel pressured to create art that is visually and technically traditional.

> Conclusion

Despite the struggles the participants consistently faced as a result of working and living in Florence, their responses repeatedly support the notion that the history of art is necessary in order to understand, create, and appreciate contemporary art; this is why it is important for Florence to move in a direction in which it celebrates both its historic art alongside its contemporary work in order to support a new generation of artistic revolutionaries.

> References

Collier, John, and Malcolm Collier. *Visual Anthropology: Photography as a Research Method*. Albuquerque: U of New Mexico, 1986. Print.

Martin, Bella, and Hanington, Bruce M. Universal Methods of Design: *100 Ways to ResearchComplex Problems, Develop Innovative Ideas, and Design Effective Solutions.* Beverly, MA: Rockport, 2012. Print.

Schwartz, Dona. "Visual Ethnography: Using Photography in Qualitative Research." *Qualitative Sociology*. 1989

Towndsend, Scott. *Re-Reading the "Toolbox:" Dourish and "Implications for Design."*

> Acknowledgments

This research was partially supported by a grant from North Carolina State University's Undergraduate Research Grant and the University Honors Program's Travel Award.

Special thanks to additional guidance provided by Scott Townsend, Associate Professor in the Department of Graphic Design and Industrial Design at North Carolina State University.

Special thanks to staff at Lorenzo de' Medici Institute in Florence, Italy.

Special thanks to Sara Amrhein and Anna Rose, the co-founders of the cultural association Creative People in Florence.

MURINE FIBROBLAST RESPONSE TO ALIGNED POLYLACTIC ACID NANOFIBERS

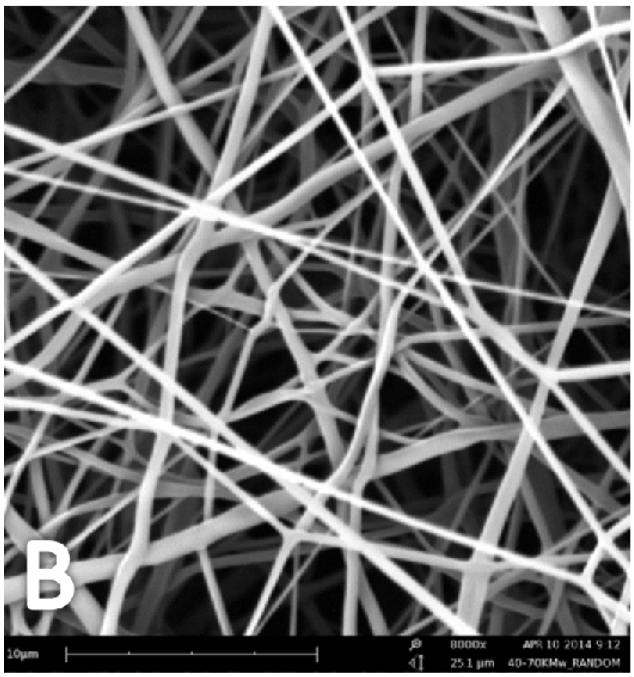


Image from a 12% solution of 40-70k Mw PLA spun onto a stationary disc.

by **Matthew Hartman**
Mentor: **Russell Gorga, Ph.D.**

Matthew Hartman graduated in May of 2015 with a B.S. in Biomedical Engineering with a minor in Tissue Engineering from NC State University and in August of 2016 with a M.S. in Biomedical Science from Wake Forest University. He is currently working as an ORAU contractor with the EPA in developing toxicological assays for nano-materials. Human health applications of biomaterials, nano-materials and structures are his primary research interest.

> Abstract

Polylactic acid (PLA) is a well-established biopolymer. It has been approved by the FDA for use in several implantable bioabsorbable implants and is a popular polymer for exploring tissue engineering techniques. Electrospinning is a processing technique which allows for polymer solutions to be "spun" into nanofibers matrices. It is theorized that topographical and mechanical properties provided by electrospun nanofibers allows for them to act as tissue scaffolds with characteristics that illicit improved attachment of cells (Chung, et al., 2009). This experiment investigated how alignment of such nanofibers made of PLA affect fibroblast growth. Aligned fibers were formed by electrospinning onto a rotating drum and unaligned fibers were spun onto a stationary surface. MTT assays and Scanning Electron Microscopy were used to compare cell growth characteristics, the focus was on the rate of cell proliferation and cellular orientation. It was found that aligned nanofibers did increase the rate of growth and there is evidence to suggest that they effect cell alignment.

> Introduction

A popuar and well established method of creating polymer based nanofibrous tissue scaffolding is electrospinning. There are three main components to the process, a pump, and high voltage source and collection plate. The pump is a device that, utilizing a disposable syringe, pumps a polymeric solution through a metal, blunt tipped needle, at a constant rate. The high voltage source usually held constant at a voltage between 5 to 20kV. The collection plate can vary in size, shape and functionality. The collection plate can be stationary or in motion. Common collection plates are flat disc shaped surfaces and cylindrical rotating drums. The voltage source is used to create an electric field between the needle tip and the collection plate, the positive end of the voltage source is connected to the needle tip and the ground is connected to the collection plate. These variables and other variables, such as distance between needle and collection plate and rate of rotation, can be controlled to control the size and orientation of the fibers (Chung, et al. 2009).

Electrospun biodegradable polymers have become very popular in the field of tissue engineering. Specifically, polylactic acid, has gained much popularity and focus because it is a material that has been approved by the FDA for implantable medical devices and electrospun PLA has been proven to be a suitable scaffold for mammalian cells (Bahattari, et al., 2006). For the purpose of this experiment a scaffold can be defined as an engineered structure to which cells are introduced and allowed to grow and proliferate throughout the structure. PLA's proven ability to be electrospun and used as a tissue scaffold is why it was chosen to be used in this experiment.

Cells are affected by their environment and often express different phenotypes based on factors such as mechanical stress, pH and orientation (Bustos, et. al, 2014). It has been observed that oriented microstructures orient some cells (Kolambkar, et al., 2014) and it has been hypothesized that orientation may increase the rate of proliferation and orientation of fibroblast cells. The orientation of cells plays an important role in the structure and function of tissues (Maninova, et al., 2014). The goal of this experiment was to see if fibroblast, a common cell type in connective tissues that often have oriented characteristics, are affected by aligned fibers. If the fibroblast cells are observed to react to topographical stimuli on the submicron scale then it suggest that the cell mechanisms for attachment and growth are stimulated by structural changes at such a scale.

A deeper understanding into cell attachment and environmental factors on cell development are cornerstones to defining proper scaffolds for tissue engineered constructs.

> Materials and Procedures

Traditional horizontal electrospinning (Ramaswamy, et al., 2011) was conducted onto two collection surfaces, one a stationary disc and the other a rotating drum. Polylactic Acid (PLA), from PolySci Corp with Mw of 40 to 70k, was electrospun at several parameters in an attempt to attain both randomly oriented fiber mats and parallel oriented fiber mats. The optimal parameters found for electrospinning the PLA were a flow rate of 35 to 50 µL/min, voltage of 10 to 20 kV and a working distance of 10 to 15 cm, combined with a 20 gauge needle. The needle used was a blunt tip 20 gauge leur lock needle from McMaster-Carr, for the production of both fiber mats. The polymer solution used for electrospinning was a mixture of 12% by weight PLA in a 3:2 mixture of Chloroform to Dimethyl Formamide, from Sigma-Aldrich, for both fiber mats. The parameters used to create fibers for this experiment were: 35µL/min flow rate, 10cm working distance, 12kV voltage source, collected on a rotating drum spinning at 7200 RPM for creation of aligned fibers. The parameters were 35µL/min flow rate, 15cm working distance, 15kV voltage source, collected on a stationary disc for creation of unaligned fibers.

The mat thickness was also controlled by spinning various amounts of polymer collecting the fiber mats and using a low force contact measuring device to measure the mats at the central area of fiber collection. From this it was determined that 0.75 mL of polymer solution needed to be spun on the stationary disc to create mats that were in the range of 0.15 and 0.2 mm in thickness and 1.75 mL of solution was necessary to create mats in the same range of thickness using the rotating drum.

Each mat had four to six samples cut from regions of the fiber mat that represented the center of collection and were examined under a Phenom Scanning Electron Microscope (SEM). For each fiber mat, at least 10 representative images were taken using the SEM. This procedure was performed in a way to ensure at least two to three pictures were taken to represent each quarter of the fiber mat sample. Using the SEM images, approximately 100 measurements of fiber diameters were made with ImageJ software, for each fiber mat. Using this data, the average fiber diameters under each processing parameters

were determined. ImageJ software was also used to analyze the surface porosity of the fiber mats, using the threshold tool. The percent surface area of empty space was determined from this procedure. The images also clearly visualized the difference in fiber orientation.

Once processing parameters were discovered that created aligned and unaligned fibers of similar fiber diameters and porosities, 8 mm diameter circles were cut from the fiber mats using a steel punch and sterilized using ethylene oxide gas. Fifteen samples of each fiber mat were cut for cell seeding with 3T3 mouse fibroblast and analysis with MTT assays. Five samples were used of each variable of alignment (aligned and unaligned), for day one, day three and day seven observations. The fiber mats and cell culture wells of the MTT assays were treated with DMSO (ATCC) to dissolve crystals and fibrous mats were removed once all color was gone from the fiber samples. The wells were then run through a UV/Vis spectrometer (BioTek Synergy UV/Vis Spectrometer) measuring absorbance at 540 nm wave length. Four 8 mm samples were also cut of each sample and seeded with 3T3 mouse fibroblast to observe at day two, under SEM (JEOL 5900LV) after glutaraldehyde (GTA) fixation in a 3% GTA in 0.1 Na Phosphate Buffer at 7.4 pH, followed by ethanol dehydration. All fibroblast cells were seeded at a density of 2×10^5 cells/cm^2 onto the sample materials and an equal number of cells were seeded into the control wells. All cells were seeded onto the fiber scaffolds after a pre-wetting of the mats were conducted by allowing ethyl alcohol to penetrate the mat followed by five washings of phosphate buffered saline (ATCC).

The cells were cultured and incubated onto the fibers using a complete growth media comprised of 89% ATCC DMEM growth media and 10% ATCC Calf Bovine Serum, and 1% Antibiotic. The 24-well plates used for scaffold seeding were Alkali Scientific Inc. 24-well Cell Culture Plates. All cultures were maintained free of contamination, and sterile operations were performed under a class II biological safety cabinet.

> Results

The aligned nanofibrous material yielded a mean surface porosity 73.5 ± 4.8% of empty space and a mean fiber diameter of 392.6 ± 136.2 nm. The unaligned nanofibrous matrerials mean surface porosity was 75.1 ± 2.9% of empty space and average fiber diameter of 364.7 ± 128.4nm were found. Further comparison of the different fiber mats can be found in Table 1 and Figure 1.

Table 1: Mean Fiber Diameter and Porosity

	Fiber Diameter (nm)		Porosity (% empty space)	
	Mean	S.D.	Mean	S.D.
Aligned	392.6	136.2	73.5	4.8
Unaligned	364.7	128.4	75.1	2.9

The table shows the mean fiber diameter in nanometers and the mean porosity as the %area of empty space on the surface of the sample, along with standard deviations.

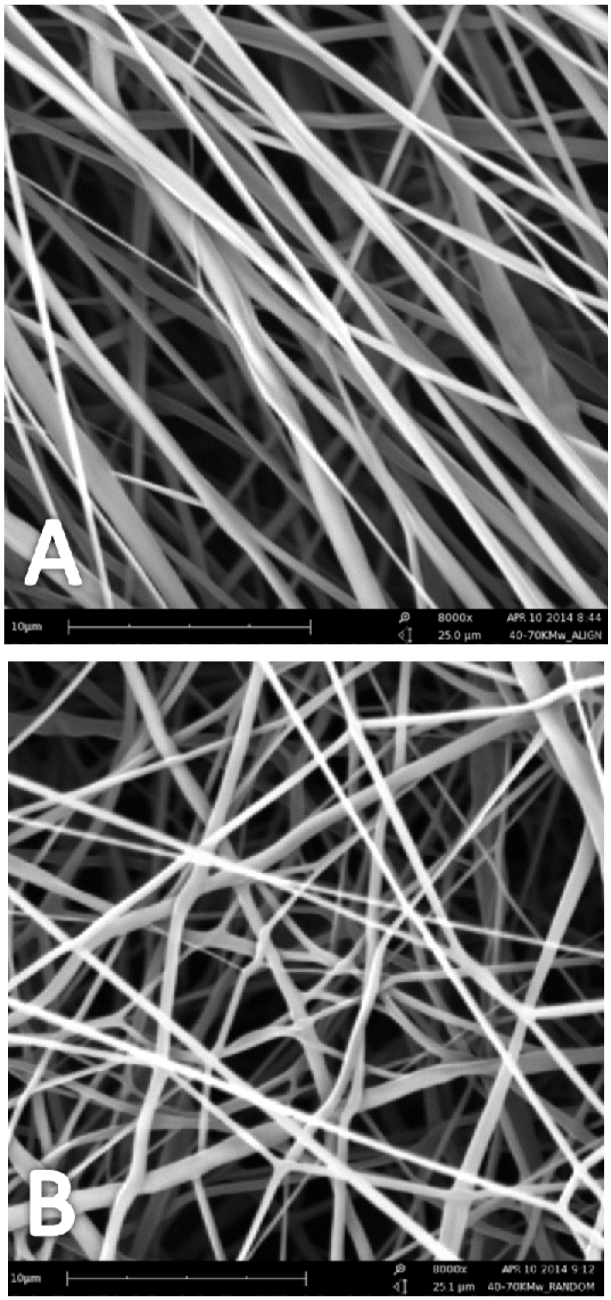


Figure 1. Image A is from a 12% solution of 40-70k Mw PLA spun onto a cylinder spinning at 7200 RPM and B is a sample of the same solution spun onto a stationary disc. Both images were taken with an SEM at 8000x. The orientation can be seen in image A, although not all of the fibers are aligned, there is a clear axis of alignment, whereas image B has no regular orientation.

The PLA nanofibers were then used as a scaffolding material for 3T3 mouse fibroblast. The growth and proliferation rate was compared using MTT assays after three and seven days. The day three results of the MTT assay indicated that there was not a significant difference between the unaligned and aligned fibers in the amount of living fibroblast. At a 90% confidence interval there was a difference between the growth and proliferation of fibroblast on the different fiber mats compared to the control wells. The statistical significance was found from a T-test indicating a P-value of 0.16 comparing aligned to unaligned, 0.071 comparing aligned fibers to the control well, and 0.021 comparing unaligned fibers to the control well. The day seven results showed a significant difference at a 95% confidence interval in the level of living cells for both fiber types to the control and for unaligned to aligned. The MTT assay indicated that the aligned fibers had an average of 23.5% more growth over a seven day period than the unaligned fibers. The significance was determined using a T-test and the P-values comparing the unaligned to aligned fibers was 0.011 (See Table 2).

The table shows the mean absorbance at 540nm of dissolved crystals from MTT assays and the P-values of T-test analyzing the difference between the samples. P-values less than 0.05 show a significant difference between two sets of data at a 95% confidence interval.

Table 2: MTT Results

	P-Values			Absorbance at 540nm		
	Aligned vs. Unaligned	Unaligned vs. Control	Aligned vs. Control	Aligned Mean	Unaligned Mean	Control Mean
Day 3	0.1599	0.0211	0.071	0.2446	0.2632	0.2052
Day 7	0.0094	0.033	0.0106	0.4012	0.3298	0.2088

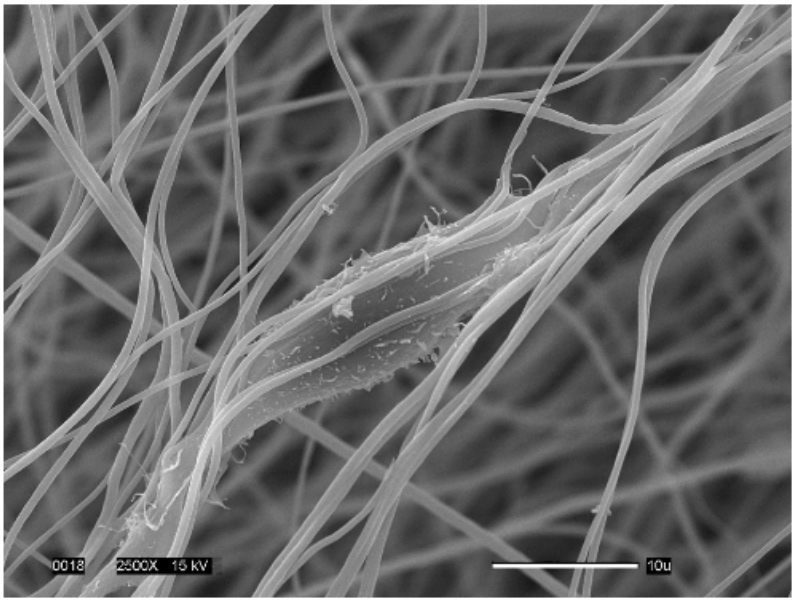


Figure 2. This is an image taken at 2500x of a relatively small fibroblast growing along a set of parallel fibers. This may indicate a response of cell response to structural stimuli.

The cells were observed to grow in an aligned fashion along the axis of orientation of the aligned fibers (Figure 2). The aligned fibers may have some effect on grouping of cells (Figures 3 and 4). It could be seen that the aligned fibers had cells randomly dispersed and they did not follow any pattern of alignment (Figures 3 and 4).

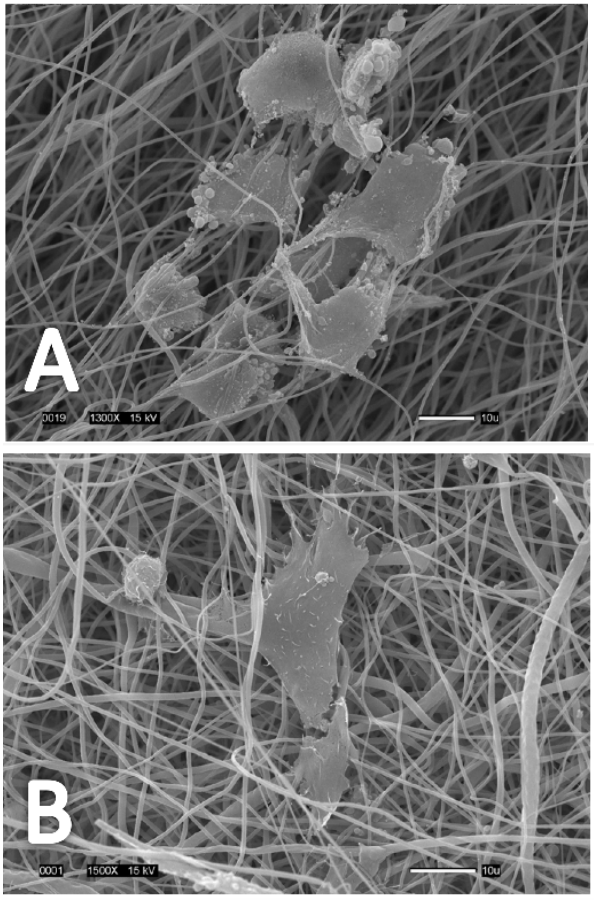


Figure 3: Image A is of several fibroblast cells on aligned fibers. It appears that the three sections in the top left of the image are one cell that broke apart. It can be seen in this image that the cells are growing along the fibers, following the orientation of said fibers. Image B is of a cell on unaligned fibers and it can be seen to be spreading out in all directions, since there is no uniformity of fibers to control cell orientation.

> Discussion

The rotating drum was successful at creating orientated fibers. The processing parameters for creating the two different types of nanofibers do vary, other than the collection method, in the voltage used and collection distance. The difference in voltage is necessary because of what is happening to the fibers during the collection onto the drum. When the fibers hit the rotating drum the polymeric fibers are stressed and the resulting strain applied to them elongates the fibers. Since PLA is a polymer the fibers undergo an isotropic elongation which decreases the fiber diameter. To create fibers of similar diameter to the fibers collected onto the stationary plate, the working voltage and collection distance were increased. This increase in voltage creates a higher magnitude electric field between the needle tip and the collection plate. This resulting electric field creates a greater applied force to the fibers as they leave the needle tip resulting in a similar higher stress and strain relationship resulting in thinner fibers. The increased distance allows for the fibers to experience the stress caused by electric field for a longer period of time, increasing the strain.

Since the two average fiber diameters are well within the standard deviations, the fiber diameters can be considered close enough to exclude any change in average fiber diameter from effecting cell growth rates. Surface porosities of the two different collection methods are also within one standard deviation of one another and can be ignored as a factor that would affect cell growth on the fiber mats. Fiber diameter, porosity and volume were maintained to best insure that the only variable that would affect cell growth was fiber orientation.

The MTT results indicated that there is an increase in late log phase growth and proliferation rate of 3T3 fibroblast cells on the aligned nanofibrous scaffold. The spectrometry results suggest an increase by approximately 23.5% compared to the unaligned fibers and 96.4% compared to the control well. These results show that the aligned nanofibers facilitate the growth and proliferation of fibroblast cells. The images taken of the cells on the scaffold material show that the fibers provide a lattice for the cell to grow along, inducing alignment of the cells (Figure 4).

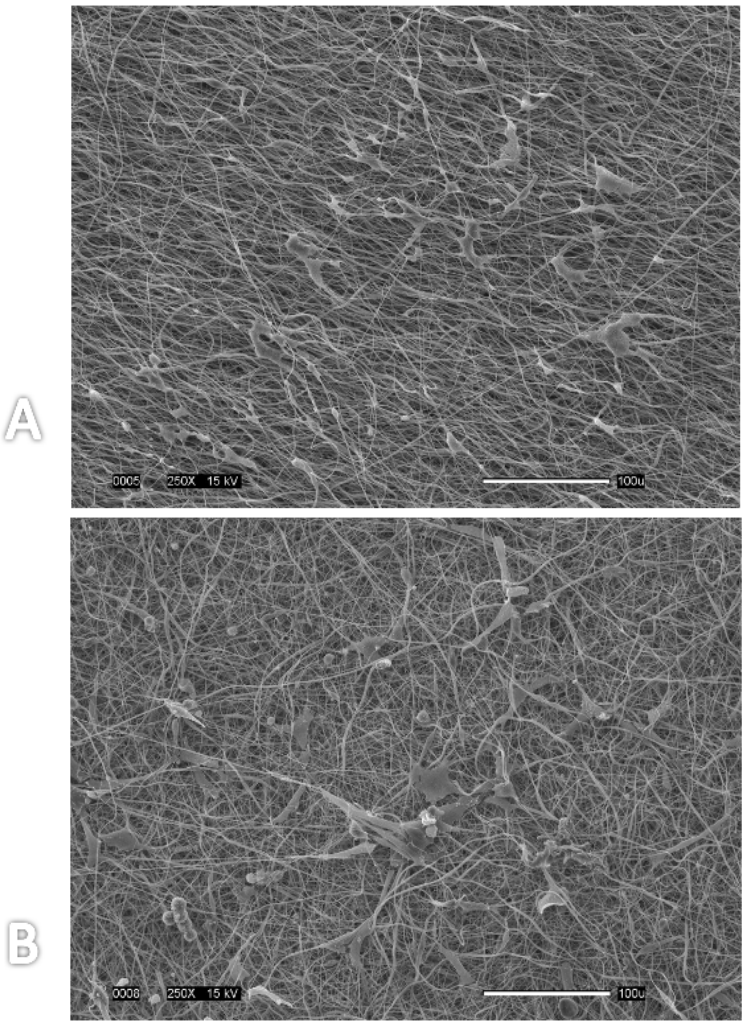


Figure 4. Image A shows fibroblast after two days of growth onto aligned PLA nanofibers.

Image B is of the same type of cells after two days of growth on unaligned PLA nanofibers. The aligned fibers seemed to orient the cells, long ways along the axis of orientation. The cells attached to the unaligned fibers were not observed to have any common orientation.

Observations of the cells on the scaffolds indicated two interesting characteristic differences between cell growths on the two fiber matrices. Observable cells on the aligned nanofiber matrix seemed smaller and more uniform in size than the cells on the unaligned nanofiber matrix, however further data would need to be collected to verify that observation. The second was that the cells on the aligned fibers seemed to be aligned with the axis of alignment of the nanofibers. Fibroblast cells reach out and expand during cell development when attached to a surface. Fibroblast cells observed in this experiment tended to have hotdog-like shapes, with one dimension of the cell being longer than the other. The cells on the unaligned fibers had no common direction of fibers around them and often reached into random directions. The cell on the aligned fibers followed the fibers around them and the orientated fibers guided them to grow along the axis of orientation. Even

when cells were turned on the aligned fibers they had a tendency to realign themselves with the axis of fiber orientation.

> Conclusion

MTT assays can indicate the difference in magnitude of cell growth of one well, or group of wells, to another. In this experiment the MTT assay indicated that the growth in the early log phase of cell growth is unaffected by the orientation of nanofibers, but the late log phase has an increased rate of growth when the cells are growing along an oriented bed of nanofibers. SEM images indicate that the fibroblast actually use the nanofibers as a sort of track to attach and spread along. The observation from the images suggest that the interaction of the nanofibers guides the fibroblast, aligning the cells if the fibers are aligned and creating random cell orientations if the cells are random.

Further experiments would be necessary to quantify and determine significant changes in the orientation of cells and other morphological characteristics observed in this experiment. The results of this experiment, did however, support previous research indicating that fibroblast like cells will align with a substrate that has an aligned surface morphology. It also confirms the hypothesis of this experiment that the growth rate of the cells is increased by the fibroblast having aligned fibers to propagate over.

Possible applications of this information could pertain towards increase the production rate of cells for bio-manufacturing purposes, the guiding of cell orientation for tissue engineering applications, and the facilitation of cell growth for wound healing applications. The increased growth and proliferation caused by alignment of fibers is a key piece of information for cell culture with fibroblast like cells. According to this experiment it is possible to facilitate growth in a way that drastically increases the production of fibroblast cells on a bio-absorbable substrate. The orientation of the cells is also particularly important, giving information to future research in possible ways to achieve the physiological cell orientations for tissue engineered products and wound healing devices. It also indicates the possibility of using either nanofibers or some other nano-structures of aligned parallel structures to increase the cell population in cultures.

> References

Bhattarai, S. R., Bhattarai, N., Viswanathamurthi, P., Yi, H. K., Hwang, P. H., & Kim, H. Y. (2006). Hydrophilic nanofibrous structure of polylactide; fabrication and cell affinity. Journal of Biomedical Materials Research Part A, 78A(2), 247-257. doi:10.1002/jbm.a.30695

Chung, S., Moghe, A. K., Montero, G. A., Kim, S. H., & King, M. W. (2009). Nanofibrous scaffolds electrospun from elastomeric biodegradable poly(L-lactide-co-epsilon-caprolactone) copolymer. Biomedical Materials, 4(1), 015019. doi:10.1088/1748-6041/4/1/015019

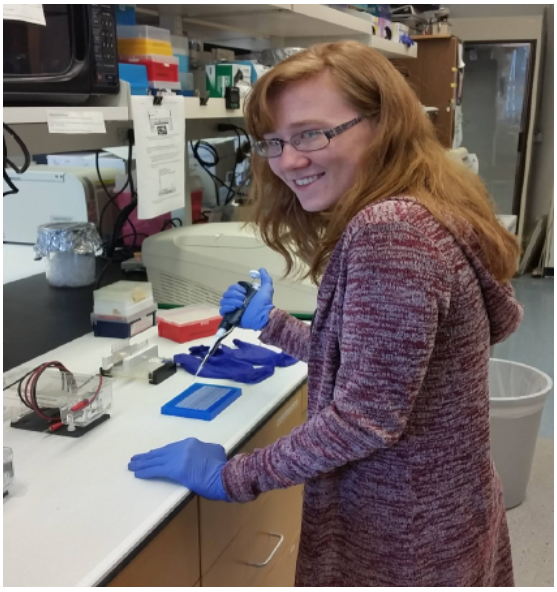
Helena Bustos, R., Suesca, E., Millan, D., Manuel Gonzalez, J., & Fontanilla, M. R. (2014). Real-time quantification of proteins secreted by artificial connective tissue made from uni- or multidirectional collagen I scaffolds and oral mucosa fibroblasts. Analytical Chemistry, 86(5), 2421-2428. doi:10.1021/ac4033164

Kolambkar, Y. M., Bajin, M., Wojtowicz, A., Hutmacher, D. W., Garcia, A. J., & Guldberg, R. E. (2014). Nanofiber orientation and surface functionalization modulate human mesenchymal stem cell behavior in vitro. Tissue Engineering Part a, 20(1-2), 398-409. doi:10.1089/ten.tea.2012.0426

Maninová, M., Iwanicki, M. P., & Vomastek, T. (2014). Emerging role for nuclear rotation and orientation in cell migration. Cell Adhesion & Migration, 8(1), 42–48. doi:10.4161/cam.27761

Sangeetha Ramaswamy, Laura I. Clarke, Russell E. Gorga, Morphological, mechanical, and electrical properties as a function of thermal bonding in electrospun nanocomposites, Polymer, 52(14), 3183-3189, doi:10.1016/j.polymer.2011.05.023.

PARTIAL CHARACTERIZATION OF A NOVEL LIPOPROTEIN, EGF LATROPHILIN AND SEVEN TRANSMEMBRANE DOMAIN CONTAINING 1 (*eltd1*) IN STRIPED BASS (*Morone saxitilis*)



by **Shelby R. Gandee**
Mentors: **Justin D. Schilling, Ph.D. & Benjamin J. Reading, Ph.D.**

Shelby R. Gandee worked for NC State University as an Undergraduate Research Technician for all three years of school, and loved every moment. Her goals in life are to continue with research, especially in animal behavior. Since graduation, Shelby now works with Clydesdale horses at Busch Gardens Williamsburg.

> Abstract

A novel estrogen responsive protein called epidermal growth factor, latrophilin, seven-transmembrane domain containing 1 protein (*eltd1*, alternatively called *adgrl4*) was identified as estrogen responsive in the blood plasma of white perch. Little information is known about *eltd1* in fishes, more specifically the striped bass – an important commercial fish. It is suggested to play an important role during reproduction by contributing to vitellogenesis through its association with vitellogenins (vtg), and can potentially serve as a biomarker of exposure to environmental estrogens such as endocrine disrupting compounds (EDCs). Current studies have demonstrated EDCs impact fish populations causing reproductive failures linked to dysregulation of vtg expression. Failure to remedy these reproductive impacts in fish species will result in continued declines of wild fish populations. For this reason, an exploratory study was conducted to verify expression of and characterize the novel estrogen responsive gene *eltd1* in striped bass (*Morone saxatilis)*. The following objectives were used to characterize *eltd1*: 1) identify the different tissue types that express *eltd1* gene transcripts in male, female, and juvenile striped bass, 2) demonstrate that *eltd1* is expressed in juvenile striped bass, 3) demonstrate how *eltd1* gene expression changes in striped bass tissues after estrogen induction, 4) perform a sequence analysis on the *eltd1* gene transcript and encoded protein, and 5) explore the genomic synteny of *eltd1* in vertebrate genomes. A partial sequence of *eltd1* was cloned out of striped bass gDNA in both male and female. However, there was no evidence of expression in the particular tissue samples used, with and without estrogen induction.

> 1. Introduction

1.1 What is *eltd1*?

Epidermal growth factor, latrophilin, seven-transmembrane domain containing 1 protein (*eltd1*, alternatively called *adgrl4*) is a novel estrogen responsive protein in vertebrates. It is approximately 690-750 amino acids in size and it belongs to the secretin family of G-protein-coupled receptors (GPCRs), which are characterized by a variable number of extracellular epidermal growth factor (EGF) repeats and seven conserved transmembrane-spanning regions (Nechiporuk et al., 2001). In vertebrates, GPCRs play a vital role in signal transduction, acting as receptors for hormones, cytokines, and other physiological stimuli (Rosenbaum et al., 2014). Adhesion GPCRs are integral membrane proteins consisting of an extracellular amino-terminus connected to seven transmembrane alpha-helical segments that form alternating intracellular and extracellular loop regions (Paavola and Hall, 2012; Rosenbaum et al., 2014). The extracellular loop regions and amino-terminus act as a reaction site, which initiates a conformational change following binding of the GPCR to a specific agonist. This change in conformation activates the heterotrimeric G protein of the GPCR, releasing it into the cell to begin the signal transduction process (Rosenbaum et al., 2014). The role GPCRs play in cell-matrix and cell-cell signal transduction is the reason nearly 30% of all available medicines in 2006 targeted GPCRs, and make G proteins an important target for future research into disease treatments (Paavola and Hall, 2012). It should be noted, however, that over 100 GPCRs are still orphans, meaning that the agonist(s) that activate the G protein remain undiscovered, and the largest subset of orphans are the adhesion GPCRs to which *eltd1* belongs. Beyond this, little information is known about the function(s) of *eltd1* in fishes, more specifically the striped bass. Information currently available about *eltd1* in other vertebrates is summarized below.

1.2 *eltd1* in cattle and mice

Although little is known about *eltd1* in fishes, some studies show a physiological role for the gene in other organisms. One study linked *eltd1* to tick burden in cattle, which can lead to major health problems in the animals (Porto Neto et al., 2011). In the study, cattle were genotyped in association with *eltd1*, which was found to be highly associated with tick burden. Heavy tick infestations can cause fever, blood loss, damage to the hide, and possible death. *ELTD1* has been linked to cancer in humans, in particular, glioblastoma multiforme (Towner et al., 2013). By using animals as research tools, scientists speculated that *eltd1* could be targeted to reduce gliomas. Mice treated with anti-*Eltd1* antibodies showed a reduction in tumor size and had an increased survival rate compared to control animals. The results indicated that the anti-*eltd1* antibody could possibly be used to treat gliomas in humans as a sole treatment or in combination with other cancer therapies.

Mice have also been used to understand the relationship between heart disease and *Eltd1*. After exposing mice with the *Eltd1* genotype and non-*Eltd1* genotype (knockouts) to ventricular pressure overloading of the heart, they found that non-*Eltd1* mice showed negative responses to the treatment (Xiao et al., 2012). These negative responses include cardiomyocyte hypertrophy, thickening of the ventricular walls, and cardiac fibrosis, thickening of the heart valves. Both cardiomyocyte hypertrophy and cardiac fibrosis are indications of reduced performance of the heart and heart failure. These heart dysfunctions are not evident following cardiac remodeling in the *Eltd1* mice. Analysis of signaling pathways in this study also revealed elevated extracellular signal-regulated kinase (ERK) and c-Jun amino-terminal kinase (JNK) phosphorylation in non-*Eltd1* mice, further supporting the role of this gene as a G-protein-coupled receptor. Much like the *Eltd1* and cancer treatment, *Eltd1* is a possible target for treating cardiac disease.

1.3 *eltd1* in humans

Recent studies have implicated the orphan GPCR, *ELTD1*, to angiogenesis, which is the formation and growth of new blood vessels. Angiogenesis is also an important factor in tumor growth and metastasis as it is the mechanism by which a developing tumor vascularizes to secure a nutrient source (Masiero et al., 2012). A recent study conducted by Masiero et al. (2012) aimed to decipher and angiogenic meta-signature of genes expressed in human tumor cells. The study illuminated a core signature of 43 overexpressed genes in common primary tumors, and of those *ELTD1* ranked the highest, with upregulation of *ELTD1* in tumor-associated endothelial cells in 89% of renal cancer samples and 82% of colorectal samples. Another study demonstrated a two- to four-fold upregulation of *ELTD1* in endothelial cell tumors (Dietrich et al., 2012). Masiero et al. (2013) also noted that higher expression of *ELTD1* in tumor-associated endothelial cells resulted in greater tumor microvascular density, a key indicator in the aggressiveness

of a particular cancer, thus further supporting the notion of *ELTD1* as an angiogenic oncogene.

The expression of the Eltd1 protein is induced by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), important cytokines in tumor angiogenesis, and suppressed by delta-like 4 (DLL4) signaling. In fact, the presence of VEGF and bFGF increased the levels of *ELTD1* mRNA and proteins in Human Umbilical Vein Endothelial Cells (HUVECs) (Masiero et al., 2013). The positive correlation between concentrations of these two proteins is a possible explanation for the upregulation of *eltd1* in tumor endothelial cells (Masiero et al., 2013), but more research is needed to affirm this claim. In subsequent experiments, the inhibition of *ELTD1* resulted in suppression of endothelial cell growth and vessel formation, which lead to decreased tumor growth *in vivo* (Masiero et al., 2013). This study implicates *ELTD1* as a possible player in tumor growth and provides a potentially rewarding avenue for further research on cancer therapies.
As mentioned before with mice, *ELTD1* has been found to be associated with high- and low-grade gliomas in humans (Towner et al., 2013). Those with high-grade gliomas had greater expression of *ELTD1* than those with low-grade gliomas. This lead to the conclusion that *ELTD1* was associated not with glioma formation, but also with the grade of the glioma (Towner et al., 2013). As with its role during angiogenesis and tumor vascularization, the suppression of *ELTD1* may prove to be beneficial in slowing the growth of multiple types of cancers, including gliomas.

1.4 *eltd1* in fishes

The *eltd1* protein remains primarily unexplored for its potential applications in fishes. The *eltd1* has been identified in other classes of organisms, including both bony fishes, such as *Danio rerio* (zebrafish), *Salmo salar* (Atlantic salmon) and *Ictalurus punctatus* (catfish). In all other fish species, the protein has only been predicted from genome sequences and has not been verified as a functional gene transcript. However, the research that identified the full-length coding sequences provided by the National Center for Biotechnology Information (NCBI) does not clarify the function of the protein in either the Atlantic salmon or the channel catfish. It is proposed that *eltd1* has a role in vitellogenesis, which could affect growth and sex differentiation of female fish.
Further search into the NCBI Expressed Sequence Tag (EST) profile of *eltd1* in zebrafish revealed the presence of the protein in several different tissues. The main sites of *eltd1* expression were the brain, eye, heart, and skin, however, only eight studies have provided this information. It does seem to correlate with the previously provided research where *eltd1* was associated with angiogenesis in the heart and in the brain. As zebrafish are more closely related to striped bass than salmon, it is expected that a similar EST profile would occur in striped bass.

1.5 Vitellogenins in fishes and their relation to *eltd1*

Vitellogenesis, the process of yolk deposition/formation, begins when environmental cues trigger the hypothalamic-pituitary-gonad axis in females. Studies have concluded these environmental cues to be temperature and photoperiod in temperate fishes such as the striped bass, which can influence levels of pituitary gonadotropins and 17-beta estradiol (E2) production by the ovarian follicle (Yuanxiang et. al., 2011). There is a release of 2 types glycoprotein gonadotropins from the pituitary gland (Pankhurst, 2008): follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH is involved in gonadal growth and development, while LH is involved in maturational events. As previously described, 17 beta-estradiol is the leading hormone responsible for vitellogenesis. Estradiol is secreted by the ovarian follicles in response to FSH, from where it is taken to the liver to bind to estrogen receptors in the hepatocyte cytoplasm (Specker and Sullivan, 1995). This initiates the synthesis of vitellogenins (Vtgs) (Pankhurst, 2008). Vtg is a glyco-phospho-lipo-protein dimer with two 200 kDa subunits (Wahli et al., 1981), which is homologous in multiple egg-laying animals including insects, fishes, and chickens (Nardelli et al., 1987; Byrne et al., 1989; Hiramatsu et al., 2002; Patino and Sullivan, 2002; Lucey, 2009). Calcium is bound to the phosphorylated parts of Vtg for skeletal development. It is then secreted into the blood by estrogen, before being absorbed by the ovaries and cleaved into lipovitellin and phosvitin, yolk proteins that will provide nutrition for the developing embryos (Wahli et al., 1981; Byrne et al., 1989; Specker and Sullivan, 1995; Hiramatsu et al., 2002; Lucey, 2009). Cathepsin D (and possibly cathepsin B) is the enzyme responsible for processing Vtg into its derivative yolk proteins (Carnevali et al., 1999; Hiramatsu et al., 2002; Lucey, 2009). Ultimately Vtg synthesis is influenced by E2 and environmental factors which stimulate the hypothalamic-pituitary gonad axis.

Oral contraceptives and hormone replacement therapies in humans often rely on the use of a

synthetic derivative of E2 known as 17alpha-ethynylestadiol (EE2) (Schilling et al., 2015). This steroid, as well as many others, may persist in human excretion and following sewage treatment and compete against endogenous E2, therefore posing a severe health risk to aquatic vertebrates (Schilling et al., 2015). Such compounds are called estrogenic endocrine disruptive chemicals (EDCs) and they may cause reproductive abnormalities, increased embryo mortality and malformation, and altered gametogenesis in fishes and amphibians (Cevasco et. al., 2008). EDCs are a growing concern not only in consideration to human fish consumption, but for maintaining fish populations in wildlife fisheries. Specifically, EE2 is a feminizing agent that can induce ovotestes (intersex or hermaphroditism) in male fishes and at low concentrations induce Vtg gene expression (Lucey, 2009). High levels of Vtg in adult male fish can cause kidney failure and other problems in blood dynamics. Vtg gene expression in male fish can be used as a molecular biomarker of exposure to estrogenic EDCs. Biomarkers are used to characterize a change in a biological response (molecular, cellular, and physiological) related to exposure to environmental chemicals. Examples of markers include plasma steroid hormones, Vtg, and gonad histology. This study aims to target Vtg and its relation to *eltd1* as a potential biomarker of estrogenic EDC exposure.

The *eltd1* protein was partially characterized by Patrick Babin (2008) in a paper discussing the conservation of Vtg gene clusters in oviparous vertebrates. Vertebrates typically have several different Vtg forms and these genes are located in different regions of the genome. Babin found that *eltd1* is located in close proximity to the Vtg gene cluster in different teleost fishes and oviparous tetrapods (Babin, 2008). Because Vtgs are a common biomarker when studying fish growth and reproduction and following exposure to estrogenic EDCs, *eltd1* also may be similarly important given its conserved association to Vtgs in the genomes of organisms.

1.6 *eltd1* in the striped bass genome and other studies

This project is aimed towards furthering research on striped bass, which is a significant aquaculture species in the United States. Farming of striped bass is estimated to be the fourth largest form of U.S. finfish aquaculture, following catfish, salmonids, and tilapia. "The goal of the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* is domestication and selective breeding to produce superior striped bass and hybrid striped bass cultivars that will enable commercial producers not only to continue bringing fish to market, but also to decrease product prices and expand the industry." Sequencing striped bass will provide information necessary for selective breeding and domestication of sustainable aquaculture species and further research on fish evolution and biology. This information was gathered from North Carolina State University's Department of Applied Ecology's Striped Bass Genome Project as led by Dr. Benjamin Reading and Dr. Charles Opperman. The genome sequence provided the target *eltd1* sequence that was used for study as presented here. Additionally, the *eltd1* gene sequence in the genome was used to identify eltd1 protein by mass spectrometry (Schilling et al., 2015).

> 2. Materials and Methods

2.1 Experimental animals and sample collection

Twenty-three samples of striped bass tissue were used from five different sample sets. Muscle, stomach, gill, and heart tissues (N=4) were collected from a juvenile striped bass (*Morone saxatilis*) to be used for tissue distribution analysis. Also used for tissue distribution were adult female tissues from the ovary, adipose tissues, heart, muscle, gut, liver, and brain (N=7). Striped bass liver tissue used in a previous study and stored at minus 80°C were used for the estrogen response analysis. The samples consisted of three striped bass livers that had been induced with estrogen at days 7, 12, and 14, and three samples from the same days that were not induced with estrogen (N=6). A second set of samples taken from the aforementioned study were two different ovary samples, a liver sample, and a blood sample (N=4) from a female striped bass that had been induced with estrogen (Williams et al., 2013). RNA extraction was done using the TRIzol Reagent® and protocol. The resulting product was nano-dropped and diluted to 10-20 ng per tissue. The RNA was then stored at -20°C until used for reverse transcriptase reactions. Two samples of gDNA, one male, and one female, provided by Benjamin Reading, who previously used them for genomic sequencing in 2011, was used. The gDNA had been stored at minus 80°C to ensure no degradation.

2.2 Primers

Two different sets of primers were designed for the gDNA and the cDNA. The gDNA primers

were designed using the full-length predicted *eltd1* sequence obtained from the striped bass genome project (Reading et al 2015). Each primer is 25bp and designed to amplify a 1200bp product. The forward primer reads 5'-AGA AGA GCG TCG GGA ACA TAC CAT C–3', and the reverse primer reads 5'-CCT CGT TGT GTC TCA GAG TGA AGG T-3'. CLUSTAL Omega alignments was done on the gDNA sequence amplified using these primers with *eltd1* that have been cloned, not predicted, out of *Danio danio* (zebrafish) and *Salmo salar* (atlantic salmon). These conserved regions on the alignment were used to design a forward and reverse primer, with a predicted amplification of around 300 bp. The forward primer read 5' –TGG AAW TGA AAC TCT WCA CKT TTG A- 3' and the reverse read 5' –TBA CTC GKG AGT TGA CAG TGA TTT C- 3'.

2.3 Amplification of partial *eltd1*

A reverse transcriptase reaction was done for the four tissue samples taken from the juvenile striped bass and the E2-induction study samples using the previously prepared RNA. The reaction was run using an Invitrogen Superscript First Strand synthesis kit and controls provided. The RT product was diluted 1:500, with the control diluted 1:1000. The dilutions was used to run a polymerase chain reaction using High Fidelity PCR SuperMix. Each reaction, cDNA and gDNA templates, will contain 1µl of template, and 1.5µl of forward primer and reverse primer each. This was totaled to 50µl with 46µl of the SuperMix. The gDNA for male and female striped bass was incubated at 94°C for two minutes to denature the template and activate the enzyme. PCR amplification ran for 35 cycles, with denaturation occurring at 94°C for 30 seconds. The annealing step was run for 30 seconds, with the temperature being set at a gradient of 52.5°C - 48°C with the control at 55°C. The extension time was 1.5 minutes at 72°C, because the target sequence was about 1,200 bp. Instructions with the primers recommended one minute of extension per every thousand base pairs. After PCR amplification, the reactions were held at 4°C until removal and stored at -20°C. The cDNA was run under the same protocol, other than the extension time. The extension time was decreased to 30 seconds, since the product size was predicted at less than 500 bp.

Each set of cDNA reactions for *eltd1* were run alongside reactions designed to amplify the *Rpl9* gene known to be present in all striped bass tissues used. This was done to ensure the RNA and resulting cDNA samples were proven viable if no product or incorrect product showed for *eltd1*.

2.4 Product analysis

Both gDNA and cDNA samples were imaged using gel electrophoresis. The band lengths were determined using DirectLoad Wide Range DNA Marker™ 50 bp - 10,000 bp. The bands were cut out of the each respective gel and sent to the NCSU Genomic Sequencing Lab. The sequences were analyzed using CLUSTAL Omega sequence alignment. The gDNA for males and females were aligned to each other to determine differences between the two sequences, and aligned to the predicted *eltd1* full-length sequence obtained from the striped bass genome to consider the accuracy of the predicted genome. The exon/intron boundaries were mapped out in the amplified gDNA sequence, and used for the cDNA analysis. Each amplified product from the cDNA tissue distribution was aligned to each other tissue, and to the predicted full-length *eltd1* sequence. The E2 study samples were also sequenced and aligned to the other known sequences, since the sample came from a different tissue, the liver.

The gDNA coding sequence was aligned to multiple organisms to determine conserved regions. All sequences were run through CLUSTAL Omega to determine the similarities of these sequences to those of other animals known to have the eltd1 protein. Organisms that have cloned regions of eltd1, such as *Homo sapian* (human), *Bos taurus* (cow), *Mus musculus* (mouse), *Rattus norvegicus* (rat), and *Xenopus laeis* (African clawed frog), were aligned to the acquired sequences to show cross-species synteny of *eltd1*. Three fish species – *Ictalarus puctatus* (channel catfish), *Salmo salar* (Atlantic salmon), and *Danio rerio* (zebrafish) – were also analyzed in conjunction with the striped bass *eltd1* sequence. A cladogram was created from the alignments for all species mentioned to show the relationships as suggested by the eltd1 protein.

> 3. Results

3.1 Genomic DNA

The gDNA electrophoresis gel showed bands for the control and for every temperature that the PCR ran under. The control band was the predicted 500 bp, while the genomic DNA products were around 1200 bp in size. Figure 1 shows the full gel run for the male and female gDNA. Due to similarity in product size at all annealing temperatures, only the bands in lane two and three were cut out and purified to send to the NCSU Genomic Sequencing Lab (GSL). The male gDNA sequence compared to the predicted sequence for *eltd1* showed almost a complete match at 1,084 bp, and the female was a complete match at 1,080 bp. Three exon regions were mapped out of the male sequence. *Figure 2* shows the aligned male sequence attained from GSL, with exon regions highlighted.

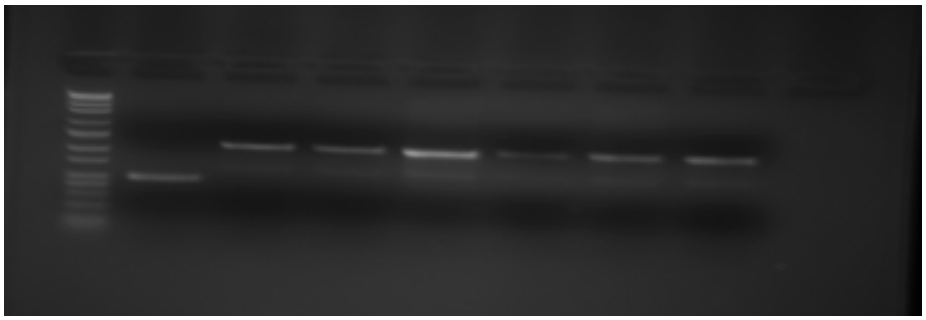


Figure 1. Gel of eltd1 PCR reactions for male and female genomic DNA. Left to right: 50-10,000 DNA marker, control reaction, male gDNA annealed at 52.5 degrees C, female annealed at 52.5 degrees C, male at 49.5 degrees C, female at 49.5 degrees C, male at 48 degrees C, and female at 48 degrees C. The control reaction (lane 1) is see at the predicted 500 bp. All genomic DNA reactions in lanes 2-7 show product around 1,200 bp.

```
Male          ------------------------------------------------------------AAGC
Msax_5086     ATGAAAGAAGAGCGTCGGGAACATACCATCACCAAGCTGCTACATACTGTTGAAGAAAGC
                                                                         ****

Male          GCTCTGACACTGGCCAACAACTACAAAACTCCAACTGAGCTCGAAATCAAAGCCAACGAA
Msax_5086     GCTCTGACACTGGCCAACAACTACAAAACTCCAACTGAGCTCGAAATCAAAGCCAACGAA
              ************************************************************

Male          ATGGGTGAGATAATTATTAACACAGAGCGCTCTTACAGACACTCAAATTATATTAAATTG
Msax_5086     ATGGGTGAGATAATTATTAACACAGAGCGCTCTTACAGACACTCAAATTATATTAAATTG
              ************************************************************

Male          TCCTGCCATTTATTCCCACCTGTGTGCAGGAAAGTGAGGTTTGCATAATTGTCATTTTAA
Msax_5086     TCCTGCCATTTATTCCCACCTGTGTGCAGGAAAGTGAGGTTTGCATAATTGTCATTTTAA
              ************************************************************

Male          TACTTTTAATTCTTTTATATACTTGATGGGGTTTTTTAATTTTTTTAATCTCAACCACAG
Msax_5086     TACTTTTAATTCTTTTATATACTTGATGGGGTTTTTTAATTTTTTTAATCTCAACCACAG
              ************************************************************

Male          AATTGAAACTCTTCACGTTTGATGCTCAACACACAAAAGCCAAAGTGTCTGCTTCCATGG
Msax_5086     AATTGAAACTCTTCACGTTTGATGCTCAACACACAAAAGCCAAAGTGTCTGCTTCCATGG
              ************************************************************

Male          CAGGAGATCACATAAATCTAACTCCAAAACTGAGACCAGAGGAGGACAGGAATGGTAAAG
```

```
Msax_5086       CAGGAGATCACATAAATCTAACTCCAAAACTGAGACCAGAGGAGGACAGGAATGGTAAAG
                ************************************************************

Male            TAGCCTAGTTTAAATGTCTAAAGGACAGTAAAATCAAAAATGCCTTTTTAAAGTTGTGAC
Msax_5086       TAGCCTAGTTTAAATGTCTAAAGGACAGTAAAATCAAAAATGCCTTTTTAAAGTTGTGAC
                ************************************************************

Male            CATCTGCATGCTTGGCACGGGGAGTACTGTTTTGCCATATGTTGCCATATGCCACATATG
Msax_5086       CATCTGCATGCTTGGCACGGGGAGTACTGTTTTGCCATATGTTGCCATATGCCACATATG
                ************************************************************

Male            TTGTACTTCAAAGTAAGTATTTGTATTTTTTTTTCTCTATCAATATATATAGCTGTAATG
Msax_5086       TTGTACTTCAAAGTAAGTATTTGTATTTTTTTTTCTCTATCAATATATATAGCTGTAATG
                ************************************************************

Male            TCTAATTTTGGGCTCGTATATTACAATTCACTCCAGTAATATCAGATTTTGGTACAAATC
Msax_5086       TCTAATTTTGGGCTCGTATATTACAATTCACTCCAGTAATATCAGATTTTGGTACAAATC
                ************************************************************

Male            TGTAAGTTTATCTGGTCATAATGGCAACATTTACACAAGCAGCAGCAGCTTGGGTCAAAA
Msax_5086       TGTAAGTTTATCTGGTCATAATGGCAACATTTACACAAGCAGCAGCAGCTTGGGTCAAAA
                ************************************************************

Male            GGTATTGGACTGTCAGCATGCAAAAAAACTGCTTCTTTATGAACTCCCATTTATAATTAA
Msax_5086       GGTATTGGACTGTCAGCATGCAAAAAAACTGCTTCTTTATGAACTCCCATTTATAATTAA
                ************************************************************

Male            ATCAAATCCAACAATTATTCTTACATTACATTATTTTATTGGGACGTTCAGTGTGTAAAC
Msax_5086       ATCAAATCCAACAATTATTCTTACATTACATTATTTTATTGGACGTTCAGTGTGGTAAAC
                ******************************************    *       ******

Male            TGGTAAGTATATACATTAAACTCTGCACAGTTGGTGAGATCTGCCAGTAGATCTAAGAGT
Msax_5086       TGGTAAGTATATACATTAAACTCTGCACAGTTGGTGAGATCTGCCAGTAGATCTAAGAGT
                ************************************************************

Male            GAAGAGCTAATTAAGTGGCTGGATTCAAAGTTTGATCACAAATACAGTAAGTTGGACTGC
Msax_5086       GAAGAGCTAATTAAGTGGCTGGATTCAAAGTTTGATCACAAATACAGTAAGTTGGACTGC
                ************************************************************

Male            CTCCCGTCTTTGTAAAACTTTTTTTGGTCCATCAGGAAGTGTGTCAGTGGTGTTTGTGCG
Msax_5086       CTCCCGTCTTTGTAAAACTTTTTTTGGTCCATCAGGAAGTGTGTCAGTGGTGTTTGTGCG
                ************************************************************

Male            ATATGACAGCATCGGTGACATCCTGAAGCCGAGCAGCGACCCAGGTGTCACCGACTACTC
Msax_5086       ATATGACAGCATCGGTGACATCCTGAAGCCGAGCAGCGACCCAGGTGTCACCGACTACTC
                ************************************************************

Male            GCGGTATGCAGGAACAGGGGAAATCACTGTCAACTCCCGAGTCATAGCAGCAGCCGTCAA
Msax_5086       GCGGTATGCAGGAACAGGGGAAATCACTGTCAACTCCCGAGTCATAGCAGCAGCCGTCAA
                ************************************************************
```

Figure 2. The genomic DNA sequence for the male striped bass aligned to the predicted full length striped bass sequence for eltd1. The male sequence is listed on the top of each line, with the predicted sequence underneath. The asterisks below each line indicate a significant match between the two sequences. The exon regions from the male gDNA are highlighted.

3.2 Complementary DNA

Out of the two tissue distributions and two E-2 induction sample sets, no conclusive products were seen in gel imaging. Some bands are seen around 50 base pairs, at the very bottom of the DNA ladder, but none was seen close to 300 base pairs, meaning there were no products in any of the 22 samples other than the *eltd1* primers. For each sample, the *Rpl9* primers produced amplified products around the predicted 300 bp, even when run at the same time as the samples with the *eltd1* primers.

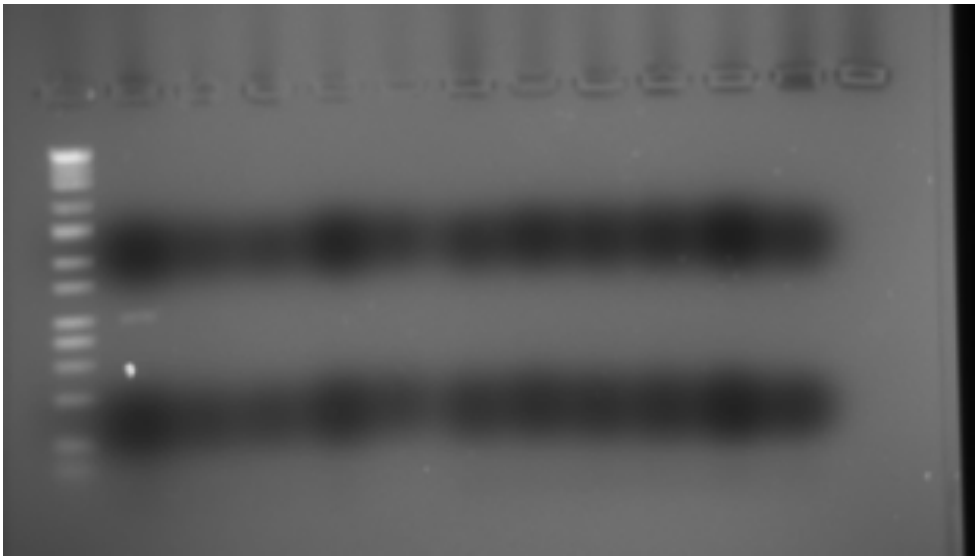
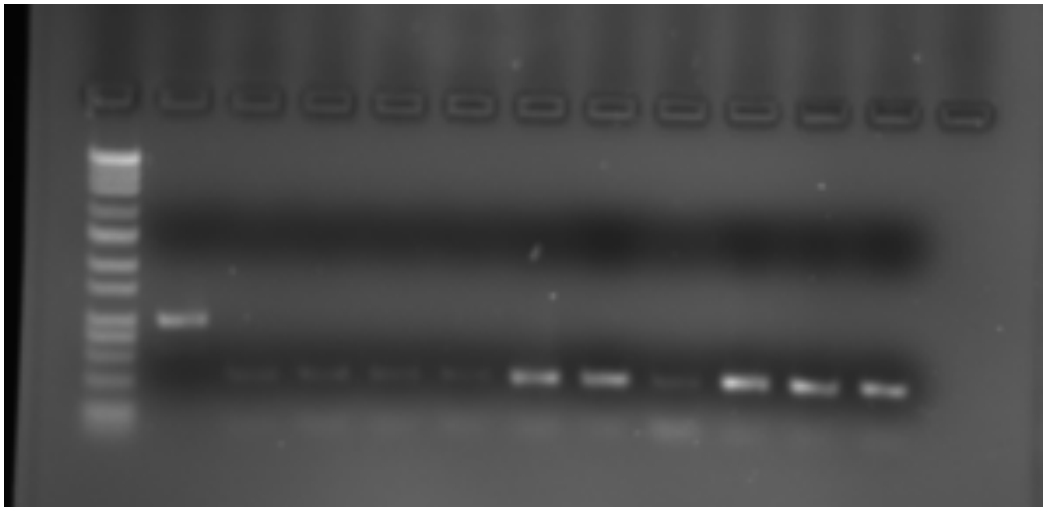


Figure 3. Gel image of juvenile striped bass tissue samples and tissue samples from an adult male liver that had been induced with estrogen using primers designed to amplify eltd1. From left to right: DNA maker, control reaction, heart, muscle, gill, stomach, control liver on day seven, E2 liver on day seven, Day 12 control liver, Day 12 E2 liver, Day 14 control liver, Day 14 E2 liver. A small band at 500 bp is seen in lane 1, representing the control reaction products. No bands are seen for any other samples.

Figure 4. Gel image of juvenile striped bass tissue samples and tissue samples from an adult male liver that had been induced with estrogen using primers designed to amplify rpl9 as a control. From left to right: DNA maker, control reaction, heart, muscle, gill, stomach, control liver on day seven, E2 liver on day seven, Day 12 control liver, Day 12 E2 liver, Day 14 control liver, Day 14 E2 liver. A small band at 500bp is seen in lane 1, representing the control reaction products. Unlike in Image 2, bands are also seen around 300bp for all samples using the rpl9 primers.



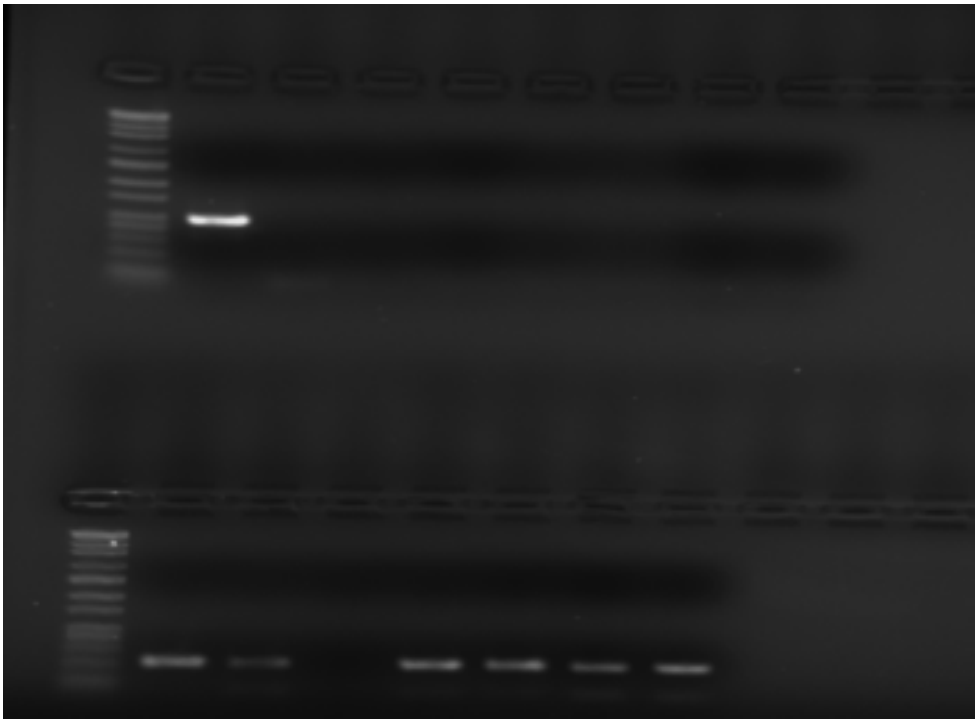


Figure 5. Gel image of PCR products for a tissue distribution of female striped bass, using samples from the ovary, adipose tissues, heart, muscle, gut, liver, and brain. The top row shows the control reaction in lane one, and the reactions using *eltd1* primers in lanes 2-8. On the bottom, lanes 1-7 show reactions using *rpl9* primers. The control reaction and *rpl9* reactions show products at the predicted size, showing the PCR worked, and the tissue samples were viable. No products were seen for *eltd1*, meaning there was no amplification of the specified sequence.

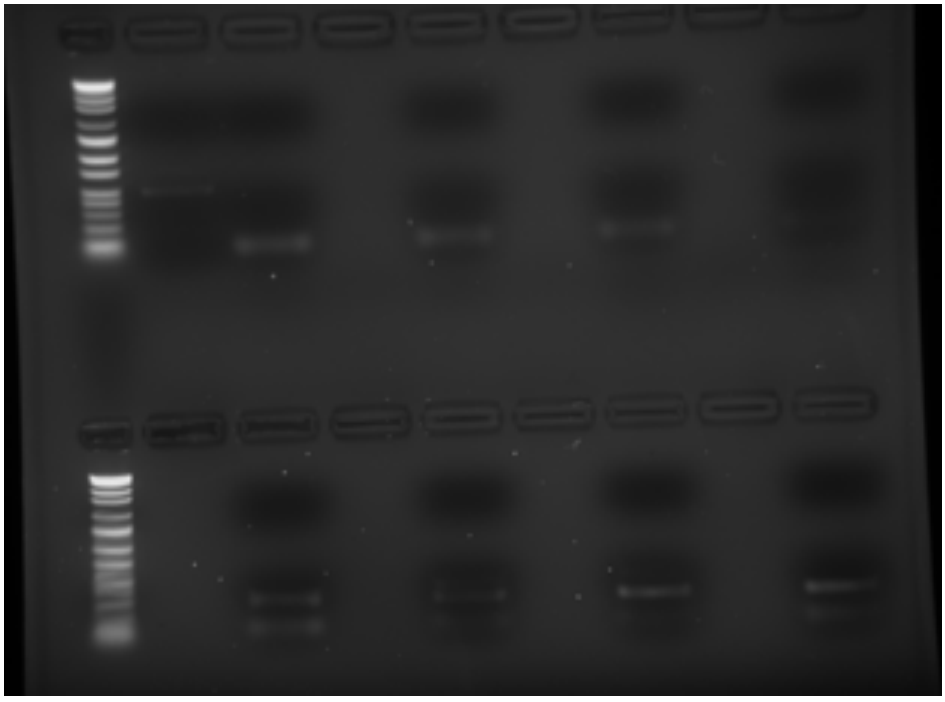


Figure 6. Gel image of estrogen induced tissues of female striped bass. Both rows from left to right: control, blood, ovary from fish #1, ovary from fish #3, and liver. The bottom row does not include a control. The top row used eltd1 primers, where the bottom row used *rpl9* primers. Amplified product for *rpl9* can be seen around 300bp, with a primer cloud less than 50bp. Bands shown for the *eltd1* are below the 50bp mark, so it is likely these are just primer clouds and not amplified product.

3.3 Homology Analysis

The mammalian and fish species used in alignments were grouped separately, resulting in a mammal only alignments and fish only alignment. Each of these two alignments were then paired with the striped bass sequence, resulting in four different CLUSTAL alignments. Only the sections that matched the partial striped bass sequence are reproduced here. The cladogram of all species with concern to *eltd1* is explained in *Figure 7*.

Mammals

```
Rattus      CACGTCACCTTACAGATCTCTCAGAACATCCAGAAGAATACTCAGTTTGACATGAATTCT
Mus         CTAGTCACCTTACAGATCGCTCAGAACACCCAGAAGAATTCTCAGTTTGATATGAATTCT
Bos         CAGGCTACCTTAAGGATATCTCAGAACTTTCAAAAGACCACTCAGTTTGATACTAATTCA
Homo        CAAGCTACTTTAAGGATATCCCAGAGCTTCCAAAAGACCACAGAGTTTGATACAAATTCA
            *: *  ** ***..***. * ****.*:  **.****. :*: ******* *  *****:

Rattus      ACCGACTTGGCTCTCAAGGTTTTCGTTTTTGATTCAGTTCACATGAAGCATACTCATCCC
Mus         ACTGACTTGGCTCTCAAGGTTTTTGCTTTTGATTCAACTCACATGAAGCATGCTCACCCC
Bos         AGTGATATAGCTCTCAAAGCTTTCTTTTTTGATTCACATCACATAAAATATATTCATCCC
Homo        ACGGATATAGCTCTCAAAGTTTTCTTTTTTGATTCATATAACATGAAACATATTCATCCT
            *  ** :*.********.* ***   **********  *.****.**. **. *** **

Rattus      CATATGAATGTGGACGGAGGCTATGTAAAAATATCCCCGAGGAGAAAATCTGCATATGAC
Mus         CACATGAATGTGGATGGAGGCTATGTGAAAATATCCCCAAGGAGAAAGGCTGCACATGGC
Bos         CATATGAATATGGATGGAGATAATATAAAGATATTTCCAAAGAGAAAAACTGCATATGAT
Homo        CATATGAATATGGATGGAGACTACATAAATATATTTCCAAAGAGAAAAGCTGCATATGAT
            ** ******.**** ****. :* .*.** ****  **.*.******. ***** ***.

Rattus      CCAAATGGCAACGTCATTGTTGCATTCCTGTGCTATAGGAGCATTGGCCCCTTGCTTTCC
Mus         ACAACTGGCAATGTAGTAGTTGCATTCCTCTGCTATAAGAGCATTGGTCCCTTGCTATCC
Bos         TCAAATGGCAGTGTTGCAGTTGCATTTTTATATTATAAGAGCATTGGTCCTCTGTTTTCA
Homo        TCAAATGGCAATGTTGCAGTTGCATTTGTATATTATAAGAGTATTGGTCCTTTGCTTTCA
             ***.*****. ** . :********  * *. ****.*** ***** **  ** *:**.

Rattus      TCATCTGACGACTTCTTACTGGGCGCTCAGAG---TGACAATTCCAAAGGAAAGGAGAAG
Mus         TCATCTGACAACTTCTTACTGGACACTCAAAA---TGATAATTCTGAAGGAAAGGAAAAA
Bos         TCATCTGACAAC---TTATTGGAACCTCAAAGTTATGATAAAGCTGAAGAAGAGAGAAGA
Homo        TCATCTGACAACTTCTTATTGAAACCTCAAAATTATGATAATTCTGAAGAGGAGGAAAGA
            *********.**   *** **... ****.*.   *** **: * .***...**...*..

Rattus      GTCATTTCTTCAGTGATTTCTGCCTCAATTAGCTCAAACCCACCCACACTGTATGAACTT
Mus         GTCATTTCTTCAGTGATTTCTGCCTCAATTAGCTCAAATCCACCCACATTATATGAACTT
Bos         GTCATCTCTTCAGTAATTTCACTCTCAATTAGCTCAAACCCACCCACATTATACGAACTT
Homo        GTCATATCTTCAGTAATTTCAGTCTCAATGAGCTCAAACCCACCCACATTATATGAACTT
            ***** ********.*****:  ****** ******** ********* *.** ******
```

INK

Mammals with striped bass

```
Mus          CTAGTCACCTTACAGATCGCTCAGAACACCCAGAAGAATTCTCAGTTTGATATGAATTCT
Rattus       CACGTCACCTTACAGATCTCTCAGAACATCCAGAAGAATACTCAGTTTGACATGAATTCT
Bos          CAGGCTACCTTAAGGATATCTCAGAACTTTCAAAAGACCACTCAGTTTGATACTAATTCA
Homo         CAAGCTACTTTAAGGATATCCCAGAGCTTCCAAAAGACCACAGAGTTTGATACAAATTCA
Morone       CTGGCCAA---------------CAACTACAAAACTCCAACTGAGCTCGAAATCAAAGCC
             *: *  *.                *.*:  .*.*. .. :*: ** * ** *  **: *

Mus          ACTGACTTGGCTCTCAAGGTTTTTGCTTTTGATTCAACTCACATGAAGC----ATGCTCA
Rattus       ACCGACTTGGCTCTCAAGGTTTTCGTTTTTGATTCAGTTCACATGAAGC----ATACTCA
Bos          AGTGATATAGCTCTCAAAGCTTTCTTTTTTGATTCACATCACATAAAAT--A--TATTCA
Homo         ACGGATATAGCTCTCAAAGTTTTCTTTTTTGATTCATATAACATGAAAC--A--TATTCA
Morone       AACGAAATGGAATTGAAACTCTTCACGTTTGATGCTCAACACACAAAAGCCAAAGTGTCT
             *  ** :*.*.: * **.   **    ****** *:  :.*** .**.         **:

Mus          CCCCCACATGAATGTGGATGGAGGCTATGTGAAAATATCCCCAAGGAGAAAGGCTGCACA
Rattus       TCCCCATATGAATGTGGACGGAGGCTATGTAAAAATATCCCCGAGGAGAAAATCTGCATA
Bos          TCCCCATATGAATATGGATGGAGATAATATAAAGATATTTCCAAAGAGAAAAACTGCATA
Homo         TCCTCATATGAATATGGATGGAGACTACATAAATATATTTCCAAAGAGAAAAGCTGCATA
Morone       GCT-------TCCATGGCAGGAGATCACATAAATCTAACTCCAAAACTGAGACCAGAGGA
              *        :. .***. ****.  * .*.** .**:  **.*... .*.. *:*.. *

Mus          TGGCACAACTGGCAATGTAGTAGTTGCATTCCTCTGCTATAAGAGCATTGGTCCCTTGCT
Rattus       TGACCCAAATGGCAACGTCATTGTTGCATTCCTGTGCTATAGGAGCATTGGCCCCTTGCT
Bos          TGATTCAAATGGCAGTGTTGCAGTTGCATTTTTATATTATAAGAGCATTGGTCCTCTGTT
Homo         TGATTCAAATGGCAATGTTGCAGTTGCATTTGTATATTATAAGAGTATTGGTCCTTTGCT
Morone       GGACAGGAATGGTAAAGTA-----------------------------------------
              *.   .*.*** *. **
```

Fish

```
Salmo          CAAACTCTTACACACAGTGGAGGAGAGTACTTTGACACTGGCCAGGAAATATAAAACAAC
Danio          CAAACTCCTTCACACAGCAGAGAAGGAAACACTGGCCCTGTCTGCTGGATACACACATGC
Ictalurus      TAAGTTACTACATACAGCAGAGCAGGGAGCAGTGGCACTTTCTAAAGCCTACAAACATTC
                **. *. *:** **** .*** **..:.*: **.*.**  * .  . .** *.*..: *

Salmo          AACTGAGATTGAGATCAAAGAGAGTGAAATGGAAATTAAACTCTACACTTTTGATGTCCA
Danio          TACTCAGATGCAGGTGCATGCCGGTGATGTAGAAATGAAATTATACACATTTGAACCCCG
Ictalurus      AGCTGAAATGCAAATCAAAACAGCTGACGTAGAACTGAAACTCTACAAATTTGTCTCCCG
               :.** *.**  *..* .*:.. . *** .*.***.* *** *.****.:****:   **.

Salmo          GCAAACTAAGAAACCCGAGCTCTCTGTCTCCATGGGAGGAGATCGTAT--CAATTTAAAT
Danio          CCAAGCACAGAAGCATCCACTGTCAGCCAACATCCAAGGAAACTCCATTTCACTAAG-CA
Ictalurus      GGAAAAACAGAAGTCCAGCATTTCAGCATCAATAGGCGGAGATTCTATTTCACTTAG-TC
                 **..:.****. .    .* **:* .:..**  ..***.*    **  **.*::.

Salmo          CCTAAA--AGGCATGA------GGGAAACAAAAATGGAAGTGTGTCCATAGTTTTCCTGC
Danio          CCAAGAA------AGCAAGACATGCAAACAACAATGGCAGTACATCCGTTGTATTCCTCA
Ictalurus      TCAAATATATGAAAGAGGAAAATACAAAC------AGGAGTGTCTCTGTTATATTCCTGC
               *:*.:       :*.       . ****      .* ***.  ** .*:.*:***** .

Salmo          GGTATGACAGTGTCGGTGGCATCCTGAAACCAAACACTGACCCTGGTGTTACAGACTACA
Danio          TCTATCACAGCATCGGGGATCTTTTAAAGCCTGCCGATGACCCTGGAGTGGCCGATTACT
Ictalurus      ACTACGACAGCATTGGGCCTCTGATGAAGCCCAAATCTGACCCCGGGGTGAACGACTATA
                **  **** .* **    .*  *.**.** ... .****** ** ** ...** ** :

Salmo          CGAGCTACGCAGAAACAGGGGAAATCACTGTGAACTCTCGGGTGATAGCGGCTGCCATCA
Danio          CTCGCTATGCAGCAGCGGGTGAAATCACAGTTAATTCACCTGTAATAGCAGCAGCCATCA
Ictalurus      GCCGATACGCCGAAGCAGGAGAGATCATAGTAAATTCTCCTGTAATAGCTGCTGCCATAA
                .*.** **.*.*.*.** **.**** :** ** **:*  **.***** **:*****.*
```

Fish with striped bass

```
Danio          GTCACCAAACTCCTTCACACAGCAGAGAAGGAAACACTGGCCCTGTCTGCTGGATACACA
Ictalurus      ATAACTAAGTTACTACATACAGCAGAGCAGGGAGCAGTGGCACTTTCTAAAGCCTACAAA
Salmo          ATAACCAAACTCTTACACACAGTGGAGGAGAGTACTTTGACACTGGCCAGGAAATATAAA
Morone         ----------------------------AGCGCTCTGACACTGGCCAACAACTACAAA
                                           .. .*: **.*.**  * .  . .** *.*

Danio          CATGCTACTCAGATGCAGGTGCATGCCGGTGATGTAGAAATGAAATTATACACATTTGAA
Ictalurus      CATTCAGCTGAAATGCAAATCAAAACAGCTGACGTAGAACTGAAACTCTACAAATTTGTC
Salmo          ACAACAACTGAGATTGAGATCAAAGAGAGTGAAATGGAAATTAAACTCTACACTTTTGAT
Morone         ACTCCAACTGAGCTCGAAATCAAAGCCAACGAAATGGAATTGAAACTCTTCACGTTTGAT
               ..: *:.** *..*  *..* .*:.. .  ** .*.*** * *** *.*:**. ****:

Danio          CCCCGCCAAGCACAGAAGCATCCACTGTCAGCCAACATCCAAGGAAACTCCATTTCACTA
Ictalurus      TCCCGGGAAAAACAGAAGTCCAGCATTTCAGCATCAATAGGCGGAGATTCTATTTCACTT
Salmo          GTCCAGCAAACTAAGAAACCCGAGCTCTCTGTCTCCATGGGAGGAGATCGTATCAATTTA
Morone         GCTCAACACACAAA---AGCCAAAGTGTCTGCTTCCATGGCAGGAGATCACATAAATCTA
                  *.  *...:.*   . .     * **:*  :..**   .***.*    ** :.: *:

Danio          AGCACCAAGAAAGCAAGACATGCAAACAACAATGGCAGTACATCCGTTGTATTCCTCATC
Ictalurus      AGTCTCAAATATATGAAAGAGGAAAATACAAACAGGAGTGTCTCTGTTATATTCCTGCAC
Salmo          AATCCTAAAA---GGCATGAGGGAAACAAAAATGGAAGTGTGTCCATAGTTTTCCTGCGG
Morone         ACTCCAAAACTGAGACCAGAGGAGGACAGGAATGGTAAAGTAGC------------CCGA
               *  .  **.     .. : * * ..* *  ** .* *.:.   *             .

Danio          TATCACAGCATCGGGGATCTTTTAAAGCCTGCCGATGACCCTGGAGTGGCCGATTACTCT
Ictalurus      TACGACAGCATTGGGCCTCTGATGAAGCCCAAATCTGACCCCGGGGGTGAACGACTATAGC
Salmo          TATGACAGTGTCGGTGGCATCCTGAAACCAAACACTGACCCTGGTGTTACAGACTACACG
Morone         TATGACAGCATCGGTGACATCCTGAAGCCGAGCAGCGACCCAGGTGTCACCGACTACTCG
               **  **** .* **    .*  *.**.** . .   ***** ** ** ...** ** :

Danio          CGCTATGCAGCAGCGGGTGAAATCACAGTTAATTCACCTGTAATAGCAGCAGCCATCAGT
Ictalurus      CGATACGCCGAAGCAGGAGAGATCATAGTAAATTCTCCTGTAATAGCTGCTGCCATAAGT
Salmo          AGCTACGCAGAAACAGGGGAAATCACTGTGAACTCTCGGGTGATAGCGGCTGCCATCAAA
Morone         CGGTATGCAGGAACAGGGGAAATCACTGTCAACTCCCGAGTCATAGCAGCAGCCGTCAA-
               .* ** **.* *.*.** **.**** :** ** ** *  ** ***** **:***.*.*.
```

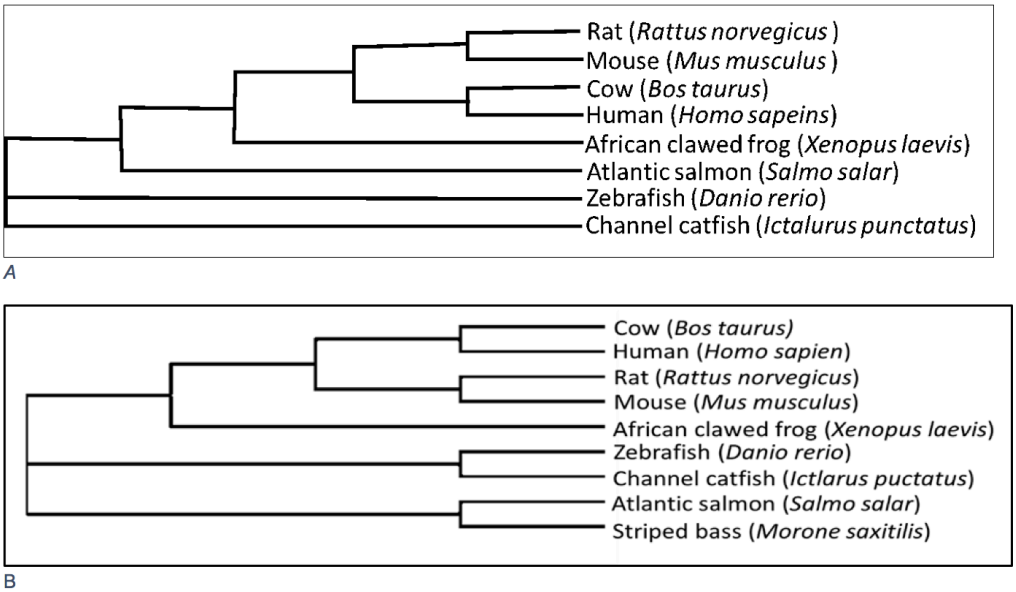


Figure 7. A) The cladogram of *ELTD1* expressed in various species shows the relationship of each species based on the full coding sequence acquired from NCBI of *ELTD,* also listed as *ADGRL4* for some species. No predicted-only sequences were used to construct the cladogram. B) Cladogram that includes the partial *eltd1* sequence obtained from striped bass, The partial sequence shows *eltd1* is more closely related in striped bass to the Atlantic salmon than to zebrafish or channel catfish.

> 4. Discussion

Current results confirm that eltd1 is present within male and femail striped bass genomic DNA. However, the study yields inconclusive results as to where and when the protein is expressed. All samples of striped bass were found viable by using Rpl9 as a control, meaning eltd1 was not expressed in the tissues used for this study.

Other measures may be taken in an attempt to find where the protein us made within striped bass. Examples of thid will be to assess the bloos plasma of a striped bass, and also the kidneys and spleen. This is because eltd1 was first found through peptide sequences in the blood found while working on the striped bass gnome project. This could mean that only red blood cells, or organs that produce red blood cells such as red bone marrow, produce the protein. The kidneys may also produce the protein along with th EPO hormone productionfor red blood cells. The striped bass that the blood was taken from had been an E2-included male and female, so the protien may not exist in any of the tissues without estrogen induction. If this is true, then male and female striped bass may induced with with estrogen and multiple tissue samples may be taken to determine where it is expressed after. This will give us more insight on how eltd1 functions within the organism, based on where it is expressed. It may also be necessart to take samples from each stage of growth in each of the aforementioned tisues in both males and females to ascertain where and when exactly the protein is expressed.

> 5. Supplementary Figures

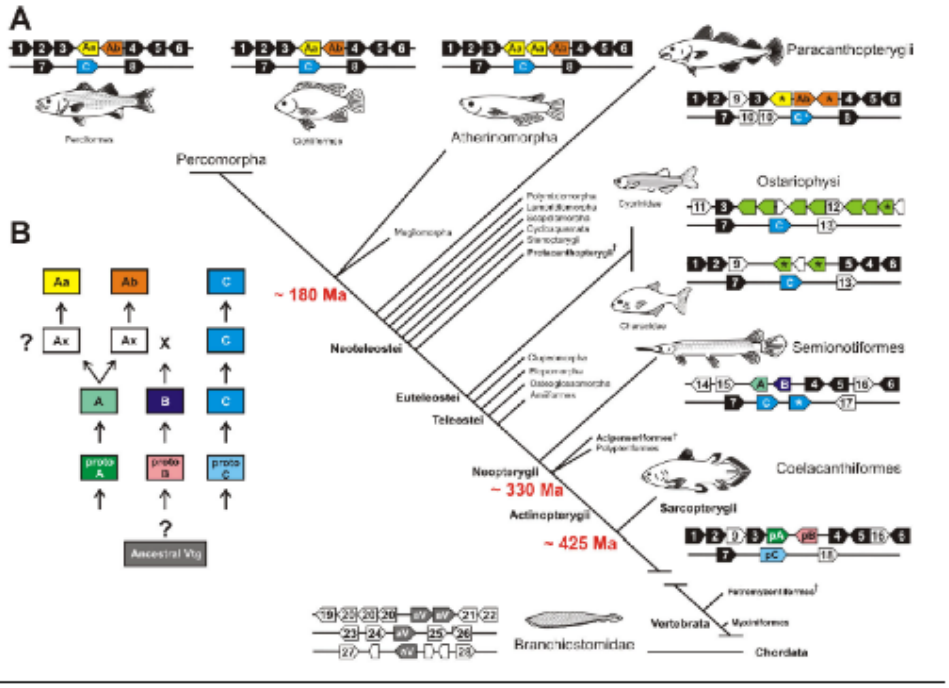


Figure 1 Genomic synteny of teleost vitellogenins (Vtgs). (A) Vtg loci were compared across species representative of taxonomic groups: Branchiostomidae (*Branchiostoma floridae*), Coelacanthiformes (*Latimeria chalumnae*), Semionotiformes (*Lepisosteus oculatus*), Ostariophysi–Cyprinidae (*Danio rerio*), Ostariophysi–Characidae (*Astyanax mexicanus*), Paracanthopterygii (*Gadus morhua*), Atherinomorpha (*Oryzias latipes*), and Percomorpha–Cichliformes (*Oreochromis niloticus*) and Percomorpha–Perciformes (*Morone saxatilis*). The arrowed boxes reflect relative strand orientation of the gene sequences, however they are not drawn to scale. Putative derived Vtg genes are classified by group colors (yellow VtGAa, orange VtgAb, blue VtgC, and purple VtgB) with black reflecting non-vitellogenin gene sequences with shared synteny to striped bass. Proto-Vtg sequences present in the Sarcopterygii and ancestral Vtg sequences present in *Branchiostoma* that gave rise to the derived Vtg forms. Empty white arrow boxes denote unidentified genes. Gene symbols are defined as: (1) *mcoln2*, (2) *lpar3*, (3) *ssx2ip*, (4) *ctbs*, (5) *spata1*, (6) *rpf1*, (7) *eltd1*, (8) *negr1*, (9) *anprc2l*, (10) *nlrc3l*, (11) *neto2*, (12) *smc5*, (13) *ifi30*, (14) *pxn*, (15) *zn692*, (16) *gng5*, (17) *cyp4b1*, (18) *ptgfr*, (19) *wls*, (20) *tmem53*, (21) *syce2*, (22) *apolp-l*, (23) *acadl*, (24) *cpt2*, (25) *lama2*, (26) *tim*, (27) *polyketide synthesis 16*, and (28) *klhl24*. Asterisks denote partial or incomplete gene predictions from the assembly. The phylogeny of fishes follows that of Nelson (1994), overlayed with reported divergence time estimates (Chen et al. 2012). (B) Hypothetical path of Vtg evolution in fishes. Vitellogenin A (VtgA), B (VtgB), and C (VtgC) arose from "proto Vtg-like" ancestors in the Actinopterygii, with the loss of VtgB occurring prior to the divergence of the Ostariophysii (Euteleostei). The *eltd1* serves as a landmark for identification of proto VtgC in Sarcopterygii. In contrast, gene duplication likely occurred in VtgA, followed by gene loss and neofunctionalization of remaining paralogs in advanced fishes (VtgAa and VtgAb).disappeared, the others remained. Followed with Figure two, this may indicate that *eltd1* branched off from a vitellogenin early on. From B.J. Reading and D.A. Baltzegar et al. (*unpublished data*).

> 5. References

Babin, P. "Conservation of a vitellogenin gene cluster in oviparous vertebrates and identification of its traces in the platypus genome." Gene 413.1 (2008): 76-82. Print

P.J. Babin, J. Cerdà, E. Lubzens (Eds.), The Fish Oocyte: from Basic Studies to Biotechnological Applications, 978-1-4020-6234-6, Springer, The Netherlands (2007), pp. 39–76.

Brander SM (2013) Thinking outside the box: Assessing endocrine disruption in aquatic life. In: Ajuha S, editor. Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation. Amsterdam, Netherlands: Elsevier.

Byrne B. M., Gruber M. and Ab G. 1989. The evolution of egg yolk proteins. Progress in Biophysics and Molecular Biology 53:33-69.

Carnevali O., Carletta R., Cambi A., Vita A. and Bromage N. 1999. Yolk formation and degradation during oocyte maturation in seabream Sparus aurata: Involvement of two lysosomal proteinases. Biology of Reproduction 60:140-146.

Alessandra Cevasco, Ralph Urbatzka, Sergio Bottero, Alessandra Massari, Federico Pedemonte, Werner Kloas, Alberta Mandich, Endocrine disrupting chemicals (EDC) with (anti)estrogenic and (anti)androgenic modes of action affecting reproductive biology of Xenopus laevis: II. Effects on gonad histomorphology, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Volume 147, Issue 2, March 2008, Pages 241-251, ISSN 1532-0456, http://dx.doi.org/10.1016/j.cbpc.2007.10.001.

Chen M, Zou M, Yang L, He S (2012) Basal jawed vertebrate phylogenomics using transcriptomic data from Solexa sequencing. PLoS One 7:e36256

D. Hanahan, R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. Cell, 144 pp. 646–674.

Hiramatsu N., Hara A., Hiramatsu K., Fukada H., Weber G. M., Denslow N. D. and Sullivan C. V. 2002a. Vitellogenin-derived yolk proteins of white perch, Morone americana: Purification, characterization, and vitellogenin-receptor binding. Biology of Reproduction 67:655-667.

Hiramatsu N., Ichikawa N., Fukada H., Fujita T., Sullivan G. V. and Hara A. 2002b. Identification and characterization of proteases involved in specific proteolysis of vitellogenin and yolk proteins in salmonids. Journal of Experimental Zoology 292:11-25.

Lucey, Sean M., "Characteristics of fish yolk proteins and a method for inducing vitellogenin" (2009). Masters Theses 1896 - February 2014. Paper 334. http://scholarworks.umass.edu/theses/334.

Masiero M, Simões FC, Han HD, Snell C, Peterkin T, Bridges E, et al. 2013. A core human primary tumor angiogenesis signature identifies the endothelial orphan receptor ELTD1 as a key regulator of angiogenesis.Cancer Cell;24: 229–241. doi: 10.1016/j.ccr.2013.06.004

Nechiporuk T, Urness LD, Keating MT. "ETL, a novel seven-transmembrane receptor that is developmentally regulated in the heart. ETL is a member of the secretin family and belongs to the epidermal growth factor-seven-transmembrane subfamily." The Journal of Biological Chemistry 276.6 (2001): 4150-4157. Print.

Nelson JS (1994) Fishes of the World. 3d ed. Wiley-Interscience, New Jersey

Paavola, K. J., & Hall, R. A. (2012). Adhesion G Protein-Coupled Receptors: Signaling, Pharmacology, and Mechanisms of Activation. Molecular Pharmacology, (5), 777–783. http://doi.org/10.1124/mol.112.080309

Pankhurst N. 2008. Gonadal steroids: function and patterns of change. 67-111 in Rocha MJ, Arukwe A and Kapoor BG editors. Fish Reproduction. Science Publishers, Enfield, NH, State. 2008

Patino R. and Sullivan C. V. 2002. Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiology and Biochemistry 26:57-70.

Porto Neto, L. R. et al. "DNA variation in the gene eltd1 is associated with tick burden in cattle." Animal genetics 42.1 (2011): 50-55. Web.

Reading, B.J., Chapman, R.W., Schaff, J.E., Scholl, E.H., Opperman, C.H., and Sullivan, C.V. 2012. An ovary transcriptome for all maturational stages of the striped bass (Morone saxatilis), a highly advanced perciform fish. BMC Research Notes 5:111.

Specker J. L. and Sullivan C. V. 1995. Vitellogenesis in fishes: status and perspectives. 304-315 in Davey KG, Peter RE and Tobe SS editors. Perspectives in Comparative Endocrinology. National Research Council of Canada, Ottawa, State. 1995

Schilling J, Nepomuceno AI, Planchart A, Yoder JA, Kelly RM, Muddiman DC2, Daniels HV, Hiramatsu N, Reading BJ. "Machine learning reveals sex-specific 17β-estradiol-responsive expression patterns in white perch (Morone americana) plasma proteins." Proteomics 15.15 (2015): 2678-90. Print.

Towner, Rheal A. et al. “eltd1, A Potential New Biomarker for Gliomas.” Neurosurgery 72.1 (2013): 77–91. PMC. Web. 28 Sept. 2015.

Wheeler, J. R., Gimeno, S., Crane, M., Lopez-Juez, E., & Morritt, D. (2005). Vitellogenin: A Review of Analytical Methods to Detect (Anti) Estrogenic Activity in Fish. Toxicology Mechanisms & Methods, 15(4), 293-306. doi:10.1080/15376520590968789.

Williams, V.N., Reading, B.J., Hiramatsu, N., Amano, H., Glassbrook, N., Islam Williams, T., and Sullivan, C.V. 2014. Multiple vitellogenins and product yolk proteins in striped bass, Morone saxatilis: molecular characterization and processing during oocyte growth and maturation. Fish Physiology and Biochemistry40(2):395-415.

Xiao, Jinfeng, et al. “Augmented cardiac hypertrophy in response to pressure overload in mice lacking eltd1.” PloS one 7.5 (2012): e35779. Web.

Yuanxiang Jin, Linjun Shu, Feiyan Huang, Limin Cao, Liwei Sun, Zhengwei Fu, Environmental cues influence EDC-mediated endocrine disruption effects in different developmental stages of Japanese medaka (Oryzias latipes), Aquatic Toxicology, Volume 101, Issue 1, 17 January 2011, Pages 254-260, ISSN 0166-445X, http://dx.doi.org/10.1016/j.aquatox.2010.10.005.

MORHPOLOGICAL COMPARISON BETWEEN ISLAND POPULATIONS OF THE HOG ISLAND BOA CONSTRICTOR

by **Nikole Sederquist & Christopher S. DePerno, Ph.D.**
Mentor: **Christopher S. DePerno, Ph.D.**
Fisheries, Wildlife, and Conservation Biology Program,
Department of Forestry and Environmental Resources,
North Carolina State University, Raleigh, NC 27695

Nikole Sederquist (formerly Nikole Tetreault) graduated from NC State University in 2014 with a Bachelor's in Fisheries, Wildlife, and Conservation Biology. She currently works at Duke University Medical Center as a Comparative Medicine Specialist.

Dr. Christopher S. DePerno is a Professor in the Fisheries, Wildlife, and Conservation Biology Program at NC State University. His research interests include population ecology, management, and habitat selection of a variety of species, animal damage management, wildlife and zoonotic diseases, and public education.

> Abstract

When organisms move from mainlands to islands, changes in body size and head morphology occur as different environmental conditions and prey types and size are encountered. However, it is unclear whether phenotypic plasticity is the reason for differences observed in snake body size and head morphology. Female snakes tend to be larger than males and may use larger prey and be subject to different selective morphological pressures. The Cayos Cochinos consists of two large islands, Cayo Cochino Mayor and Cayo Cochino Menor, which are located off the coast of Honduras. The Hog Island boa constrictors (Boa constrictor imperator) that reside on the islands are smaller compared to individuals on mainland Honduras. However, it is unclear if morphology differences exist between Hog Island boa constrictor populations on the Cayos Cochinos. The mammalian species complex differs between the islands with paca (Agouti paca), agouti (Dasyprocta punctata), and armadillo (Dasypus novemcintus) present on Cayo Cochino Mayor, but absent on Cayo Cochino Menor which may exert different selective morphological pressures. During July –August 2012, we captured boas and measured snout vent length and tail length and head morphology measurements including length, head, labial, ocular, rostral-ocular, nares-ocular, internares, and interocular. We captured 72 snakes (48 males, 24 females); 46 were captured on Cayo Cochino Menor (30 males, 16 females) and 26 were captured on Cayo Cochino Mayor (18 males, 8 females). Nearly all measurements were larger on Cayo Cochino Mayor than on Cayo Cochino Menor and nearly all measurements were larger in females than males. Nearly all measurements were larger in females than males from Cayo Cochino Mayor and females and males from Cayo Cochino Menor. Although the mechanistic pressures between the islands are unclear, we hypothesize that prey availability and size may have exerted evolutionary pressures on the isolated population driving head morphology and differences in body size.

> Introduction

When organisms move from the mainland to islands, changes in body size and head morphology occur as different environmental conditions and prey types are encountered (Boback 2003, McClain et al. 2013). Further, morphological changes in response to the environment may be adaptive or phenotypic plastic (Boback 2003). Adaptation refers to the changes in genes that occur when an organism adjusts to the new environment, while phenotypic plasticity refers to the differences in phenotypes that may occur due to different environmental conditions (Conover and Schultz 1995). For example, differences in prey type caused differences in the trophic structure due to phenotypic plasticity in fishes and mammals (Myers et al. 1996, Meyer 1987). Similarly, changes in prey type are believed to be responsible for the body size differences between mainland and island snake species (Queral-Regil and King 1998). Additionally, differences in trophic structure and prey size may cause genetic changes over time (Aubret et al. 2004). However, it is unclear whether phenotypic plasticity is the reason for differences observed in snake body size and head morphology (Forsman and Shine 1997).
Male and female snakes may develop different morphological characteristics (Shine 1991). Female snakes tend to be larger than male counterparts to maximize their reproductive output, except in species where male to male combat is common (Shine 1994). Because of the larger size, females may use larger prey and be subject to different selective morphological pressures (Queral-Regil and King 1998).

The Cayos Cochinos consists of two large islands, Cayo Cochino Mayor and Cayo Cochino Menor, which are located off the coast of Honduras (Figure 1). The Hog Island boa constrictor (*Boa constrictor imperator*) is an insular dwarfed race of boa constrictor. The island snakes are much smaller compared to individuals on mainland Honduras and are popular in the pet trade due to their size, light coloration, and docile demeanor and have been shown to differ at the microsatellite level (Green 2010). Because of the limited gene flow between Honduras and the two islands, environmental and genetic factors may have exerted different evolutionary pressures on the two populations of the Hog Island boa constrictor. Also, the mammalian species complex differs between the islands, which may influence prey selection. Therefore, our objective was to determine if head morphology differences exist between Hog Island boa constrictor populations on the Cayos Cochinos.

> Study Sites

The Cayos Cochinos were a group of islands off of the Atlantic coast of mainland Honduras. Cayo Cochino Menor and Cayo Cochino Mayor were the two largest islands (Figure 1). Cayo Cochino Menor was 65 ha in size with an elevation of 140 m above sea level and Cayo Cochino Mayor was 165 ha with an elevation of 142 m above sea level (Bermingham et al. 1998). The two islands were dominated by oak forest (*Quercus,* spp.) but the composition of the understory differed due to water availability (Bermingham et al. 1998). Fresh water was available year round on Cayo Cochino Mayor but only seasonally on Cayo Cochino Menor (Green 2010). There was a distinct rainy and dry season with the rainy season lasting from October to January, and the dry season from February to September. The topography of the islands were similar with Cayo Cochino Mayor having steeper terrain. Species diversity was higher on the Cayo Cochino Mayor (Bermingham et al. 1998). Cayo Cochino Menor was uninhabited by people except for a research station, whereas Cayo Cochino Mayor was home to a small Garifuna village, a dive resort and several vacation homes (Green 2010).

> Methods

From 12 July – 13 August 2012, we conducted localized opportunistic searches on Cayo Cochino Minor and Cayo Cochino Mayor. We conducted daily visual encounter surveys (VES) on Cayo Cochino Menor and sampled Cayo Cochino Mayor once per week. Visual encounter surveys were usually conducted in the morning and afternoon, with some occurring during the evening. We captured boas by hand when encountered and processed most snakes in the field, however, some were processed at base camp and returned to place of capture within 24 hours. We measured snout vent length and tail length using a flexible measuring tape and weighed snakes using a spring scale (Green 2010). We collected head morphology measurements (i.e. head length, head width, labial, ocular, rostral-ocular, nares-ocular, internares, and interocular) using digital calipers (Boback 2006). We compared morphological measurements between islands and tested for an island, sex, and/or island*sex effect using ANOVA.

> Results

From 12 July – 13 August 2012, we captured 72 snakes (48 males, 24 females); 46 snakes were captured on Cayo Cochino Menor (30 males, 16 females) and 26 snakes were captured on Cayo Cochino Mayor (18

larger on Cayo Cochino Mayor than on Cayo Cochino Menor with the exception of tail length and ocular (Table 1). When compared by sex all morphological measurements were larger in females than males, with the exception of tail length, which was larger in males, and ocular which was similar between the sexes (Table 2). When compared by sex and between the islands, all variables were larger in females from Cayo Cochino Mayor than males from Cayo Cochino Mayor and females and males from Cayo Cochino Menor with the exception of tail length, which was similar by sex and island (Table 3).

> Discussion

Based on our results, the females on Cayo Cochino Mayor were larger than males and females on Cayo Cochino Menor. It is believed that Cayo Cochino Mayor and Menor diverged about 5,000 years ago (Green 2010). Although the mechanistic pressures between the islands are unclear, we hypothesize that prey availability, prey size, and island area may have an effect on the morphological differences between the islands. We observed anecdotal evidence indicating that paca (*Agouti paca*), agouti (*Dasyprocta punctata*), and armadillo (*Dasypus novemcintus*) were present on Cayo Cochino Mayor, but absent on Cayo Cochino Menor. For example, Bermingham et al. (1995) described agouti as being prevalent on both islands; however, no sightings of agouti have occurred on Cayo Cochino Menor in the last 5-7 years. Availability of prey types could explain the differences in head morphological measurements. Additionally, the larger prey on Cayo Cochino Mayor may be exerting pressures on the isolated population and driving head morphology and differences in body size between the islands (Forsman 1991). Also, the differences in the area of the islands could explain the differences in body size and head morphology. On smaller islands, boa sizes are less variable and there is a tendency of snakes to obtain a body size of 1.0 meter (Boback and Guyer 2003). Nevertheless, future research is necessary to evaluate prey abundance, prey size, and island area to further evaluate the factors that may be driving the differences in head morphology between distinct populations of the Hog Island boa constrictor.

> Acknowledgments

We thank Operation Wallacea and the Fisheries, Wildlife, and Conservation Biology Program at North Carolina State University.

> Literature Cited

Aubret F., R. Shine, and X. Bonnet. 2004. Adaptive developmental plasticity in snakes. *Nature* 431: 261-262.

Bermingham E., A. Coates, G. Cruz, L. Emmons, R. B. Foster, R. Leschen, G. Seutin, S. Thorn, W. Wcislo, and B. Werfel. 1998. Geology and terrestrial flora and fauna of Cayos Cochinos, Honduras. *Revista De Biologia Tropical* 46: 15-37.

Boback, S. M. 2003. Body size evolution in snakes: evidence from island populations. *Copeia* 2003:81-94.

Boback, S. M. 2006. A morphometric comparison of island and mainland boas (*Boa constrictor*) in Belize. *Copeia* 2006: 261-267.

Boback, S. M., and C. Guyer. 2003. Empirical evidence for optimal body size in snakes. *Evolution* 57: 345-351.

Conover, O. C., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* 10:248-252.

Green, S. 2010. Evolutionary biology and conservation of the Hog Island boa constrictor. Ph.D. thesis. University of Kent, UK.

Forsman, A. 1991. Variation in sexual size dimorphism and maximum body size among adder populations: effects of prey size. *Journal of Animal Ecology* 60: 253-267.

Forsman, A., and R. Shine. 1997. Rejection of non-adaptive hypotheses for intraspecific variation in tropic morphology in gape-limited predators. *Biological Journal of the Linnean Society* 62: 209-223.

McClain, C. R., P. A. P. Durst, A. G. Boyer, and C. D. Francis. 2013. Unraveling the determinants of insular body size shifts. *Biology Letters* 9: 20120989. http://dx.doi.org/10.1098/rsbl.2012.0989

Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichilidae) and their implications for speciation in cichlid fishes. *Evolution* 41: 1357-1369.

Myers P., B. L. Lundrigan, B. W. Gillespie, and M. L. Zelditch. 1996. Phenotypic plasticity in skull and dental morphology in the prairie deer mouse (*Peromyscus maniculatus bairdii). Journal of Morphology* 229: 229-237.

Queral-Regil, A., and R. B. King. 1998. Evidence for phenotypic plasticity in snake body size and relative head dimensions in response to amount and size of prey. *Copeia* 1998: 423-429.

Shine, R. 1991. Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. *The American Naturalist* 138:103-122.

Shine, R. 1994. Sexual size dimorphism in snakes revisited. *Copeia* 1994: 326-346.

> Tables and Figures

Table 1. Morphological comparisons between Cayo Cochino Mayor and Menor of the Hog Island boa constrictor, 2012.

Measurement	Island Mayor (n= 26)		Island Menor (n=46)		F-value	P-value
	Mean	SE	Mean	SE		
Snout vent length (cm)	101.2	4.9	88.6	2.9	11.64	0.001
Tail length (cm)	15.4	0.9	13.7	0.6	3.78	0.056
Mass (g)	563.5	117.7	370.4	35.1	10.59	0.002
Head length (mm)	42.3	1.8	38.4	0.9	15.43	<0.001
Head width (mm)	25.7	1.5	22.6	0.6	15.26	<0.001
Labial (mm)	32.5	1.5	28.7	0.7	17.99	<0.001
Ocular (mm)	5.4	0.1	5.4	0.1	0.98	0.325
Naresocular (mm)	12.2	0.6	10.7	0.3	15.99	<0.001
Rostral-ocular (mm)	15.6	0.7	13.7	0.4	19.70	<0.001
Internares (mm)	6.9	0.3	6.6	0.2	6.65	0.012
Interocular (mm)	15.5	0.6	14.4	0.3	11.93	0.001

Table 2. Morphological comparisons by sex of the Hog Island boa constrictor, Cayo Cochino Mayor and Menor, 2012.

Measurement	Island Mayor (n= 26)		Island Menor (n=46)		F-value	P-value
	Mean	SE	Mean	SE		
Snout vent length (cm)	99.1	6.2	90.2	2.4	6.85	0.0011
Tail length (cm)	12.0	0.7	15.5	0.6	8.75	0.004
Mass (g)	649.8	131.5	335.3	21.6	18.89	<0.001
Head length (mm)	43.8	2.1	37.8	0.7	24.25	<0.001
Head width (mm)	26.2	1.6	22.5	0.5	17.14	<0.001
Labial (mm)	33.0	1.7	28.6	0.6	20.01	<0.001
Ocular (mm)	5.4	0.2	5.4	0.1	1.34	0.251
Naresocular (mm)	12.6	0.6	10.6	0.3	23.30	<0.001
Rostral-ocular (mm)	16.1	0.8	13.6	0.3	27.02	<0.001
Internares (mm)	7.3	0.3	6.4	0.1	14.51	<0.001
Interocular (mm)	15.6	0.7	14.4	0.3	12.87	0.001

Table 3. Morphological comparisons of the Hog Island boa constrictor by sex and between Cayo Cochino Mayor and Menor, 2012.

Measurement	Female Mayor (n=8)		Female Menor (n=16)		Male Mayor (n=18)		Male Menor (n=30)		F-value	P-value
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Snout vent length (cm)	121.5	10.0	87.9	6.4	92.2	4.2	89.0	3.0	8.03	0.006
Tail length (cm)	14.1	0.8	11.0	0.8	16.0	1.2	15.2	0.7	1.25	0.268
Mass (g)	1042.5	325.8	453.4	85.4	350.6	36.6	326.2	27.0	8.98	0.004
Head length (mm)	52.2	3.1	39.6	2.0	38.0	1.2	37.7	0.9	14.22	<0.001
Head width (mm)	32.7	3.1	22.9	1.3	22.6	0.9	22.4	0.6	14.03	<0.001
Labial (mm)	40.0	3.1	29.5	1.5	29.1	0.9	28.3	0.7	13.01	0.001
Ocular (mm)	5.9	0.2	5.2	0.2	5.2	0.2	5.5	0.1	8.86	0.004
Naresocular (mm)	15.1	0.9	11.4	0.6	10.9	0.4	10.3	0.3	8.32	0.005
Rostral-ocular (mm)	19.6	1.0	14.3	0.7	13.9	0.6	13.4	0.4	13.78	<0.001
Internares (mm)	8.4	0.5	6.7	0.4	6.3	0.2	6.5	0.2	10.77	0.002
Interocular (mm)	18.3	1.1	14.2	0.6	14.2	0.4	14.5	0.3	16.31	<0.001

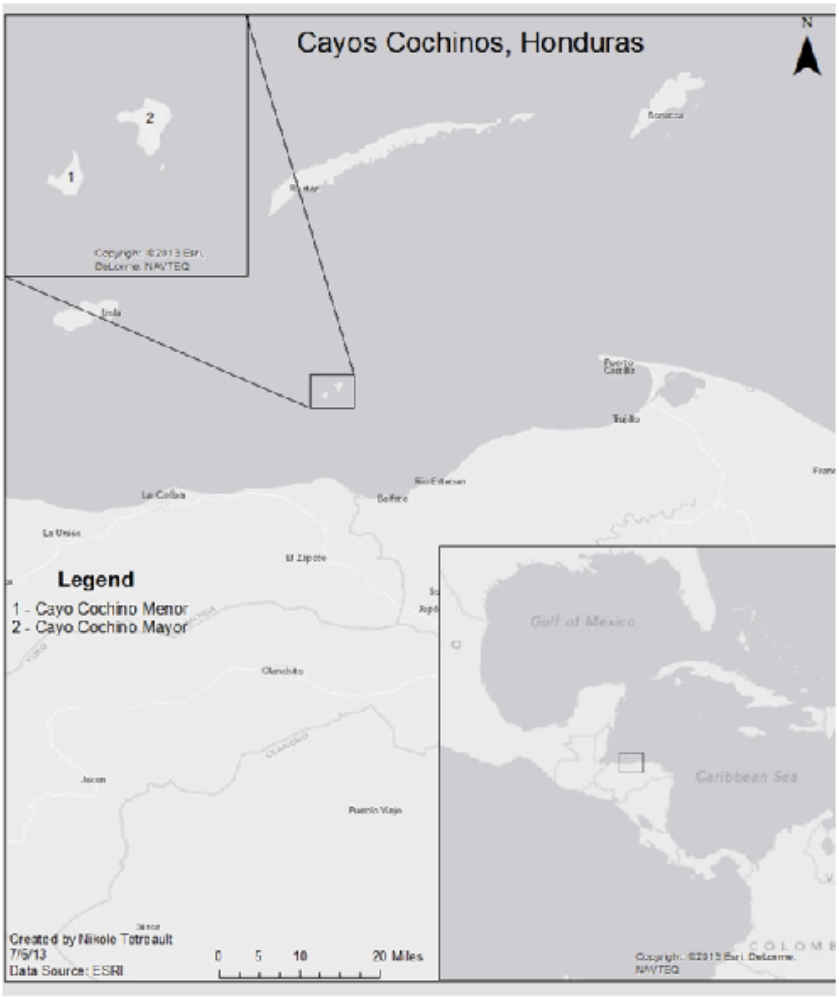
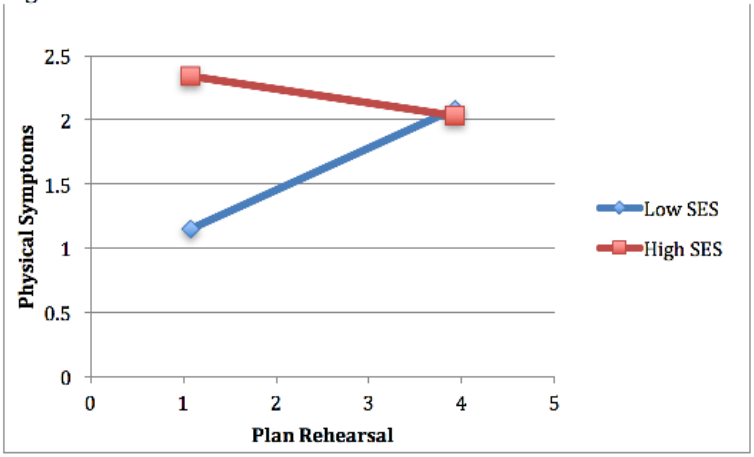


Figure 1. The Cayos Cochinos Islands, Honduras.

MORE MONEY, MORE PROBLEMS? SUBJECTIVE SOCIOECONOMIC STATUS, COPING, AND DAILY PHYSICAL HEALTH

Figure 1.



Number of physical health symptoms experienced in older adults in relation to plan rehearsal coping, and depending on high or low SES.

by **Anna Fisher**
Mentor: **Shevaun D. Neupert, Ph.D.**

Anna Fisher graduated with a B.A. in psychology and a biology minor from NC State University. During college, she was a member of Delta Delta Delta and an athlete on the competitive cheerleading team. She is currently working as a recruiter for an Information Technology staffing firm in Raleigh, NC.

> Abstract

Coping efforts may play a role in older adults' daily life by affecting the number of physical health symptoms they experience each day. Anticipatory coping is a form of coping that helps prepare for future stressful events, and we focused on preparing for arguments. We looked at physical health symptoms on a daily basis and examined whether subjective socioeconomic status (SES) rankings interacted with four forms of anticipatory coping. Using daily diary data from 43 older adults across 8 consecutive days, multilevel models yielded different results. Plan rehearsal appears to benefit people who view themselves as having fewer resources than others, but negatively impacts those who view themselves as having more resources. In contrast, stagnant deliberation and fantasy outcome are consistent with our hypothesis that the coping efforts have a positive effect on all participants for both high and low self-ranked SES.

> Introduction

Stress is a cause of physical health suffering in older adults, mainly due to outside stressors that create tension and force the body to adapt (Mallers, Almeida, & Neupert, 2004). This study examines the effects of two external factors, subjective socioeconomic status (SES) and anticipatory coping, on older adults' physical health. Adults between the ages of 60-96 are the focus of the study because older adults comprise the largestage-group of people who experience minor daily health symptoms, which occur much more frequently than serious conditions such as multiple sclerosis (Larsen & Kasimatis, 1991; Mallers et al., 2004). These minor symptoms, which include headaches, musculoskeletal problems, body aches, fatigue, and chest pain (Verbrugge, 1986; 1989) should not be overlooked because they disrupt daily function and are associated with circulatory diseases (Verbrugge, 1986).

Older adults undergo many major life events, most of which are related to role changes and losses. These events, such as children leaving the nest, loss of family and friends, and retirement (Lowenthal et al., 1975; Rossi, 1980), place stress on the individuals and predict a higher risk for physical, mental, and social dysfunctions (Mallers, Almeida, & Neupert, 2004). Research by Lazarus and Folkman (1984) reveals that these major life stressors do not have as much influence on physical health in older adults as day-to-day stressors do. Daily stressors are more influential due to their cumulative separate and direct effects on health (Kanner et al., 1981).

Anticipatory coping is a mental process that minimizes the impact of stressful events, and takes place at the stage of coping prior to experiencing the stressful event (Feldman & Hayes, 2005). The cognitive form of anticipatory coping, which precedes and accompanies the behavioral form, serves the purpose of developing strategies to solve problems and grasping a better understanding of one's self and circumstances by searching out new information (Schwarzer & Knoll, 2003; Segerstrom, Stanton, Alden, & Shortridge, 2003; Taylor, 1983). Plan rehearsal is a specific form of anticipatory coping that involves mentally envisioning the steps or strategies that could be used to achieve a desired outcome in order to prepare for a stressful event (Taylor & Schneider, 1989). This is particularly effective when preparing for acute, stressful tasks or big life transitions because it provides a mean for maximizing performance of coping efforts made at first and also managing these known risks (Aspinwall & Taylor, 1997; Schwarzer & Knoll, 2003). Previous studies have shown that plan rehearsal is effective at regulating emotions and attaining goals (Taylor et al. 1998). Mental rehearsal and covert modeling, both forms of plan rehearsal, have been used in other areas such as improving sports performance (Suinn, 1997), preventing substance use relapse (Marlatt & Gordon, 1985), premature termination of therapy (Sherman & Anderson, 1987), preparing depressed individuals for behavioral activation (Beck, Rush, Shaw & Emery, 1979; Jacobson et al., 1996), and teaching adaptive coping to individuals diagnosed with borderline personality disorder (Linehan, 1993). In the present study, anticipatory coping is measured with an adapted version of the MMAP (Feldman & Hayes), designed to capture day-to-day changes within a person, across stressor contexts. One important adaption of this questionnaire was to make it more context-specific; we asked participants to think ahead to specific stressful events and then answer the anticipatory coping questions. A distinguishing characteristic of this study is that it focused on the specific stage of coping prior to the experience of the stressful event. Many other questionnaires neglect to focus on a specific stage of coping, which is important to do since they affect other stages of the coping process.

Problem analysis is another form of adaptive coping. This method requires first making sense of a potential future stressful situation, and then analyzing preceding events to help cope and plan for the stressful situation (Aspinwall & Taylor, 1997). This technique has been used previously in clinical interventions for effective problem solving (D'Zurilla & Nezu, 2001) and other kinds of psychotherapy that focus on targeting a problem (Stiles et al., 1990).

Unlike planning and problem analysis, worry and rumination are typically not as effective in terms of coping (Feldman & Hayes, 2005). Stagnant deliberation involves obsessively dwelling on a stressful problem while not advancing towards a solution. This deliberation causes negative expectancies which could lead to the self-fulfilling prophecy effect (Armor & Taylor, 2003). Ongoing duration of this type of rumination is associated with poorer cognitive flexibility (Davis & Nolen-Hoeksema, 2000), as well as a longer time experiencing symptoms of mental disorders like depression and anxiety (Borkovec, 1994; Mor & Winquist, 2002; Nolen-Hoeksema, 2000). Those disorders arise from the inability to disengage from unproductive thoughts (Abramson et al., 2002; Pyszczynski & Greenberg, 1987). Importantly, these previous studies

examined stagnant deliberation from a between-person perspective; that is, differences in coping (non) effectiveness was compared across individuals. In the current study, we take a dynamic and contextual approach where we look at the effects of stagnant deliberation as it may change from day-to-day and across stressor contexts.

Outcome fantasy is the fourth anticipatory coping process. Individuals who experience outcome fantasy often daydream or fantasize about a desired outcome to a potential stressful event. Like stagnant deliberation, it is typically considered an unproductive thought process because it ignores details critical to problem solving (Feldman & Hayes, 2005). The result of this is poorer emotion regulation and goal attainment (Taylor et al., 1998), most likely because effort invested in reaching a goal is reduced when an individual engages in outcome fantasy (Oettingen, 1996). Just like stagnant deliberation, previous findings with outcome fantasy have not considered dynamic and contextual experiences, as we do here.

Subjective socioeconomic status is assessed in terms of respondents' ratings of their own perceived social class. It usually involves a self-rating of factors regarding money, education, and occupation (Adler, Epel, Castellazzo, & Ickovics, 2000). This differs from objective SES, which uses more direct measures and does not rely on self-ratings. Subjective SES has previously been identified as a closer stress-marker for older Americans than objective SES, indicated by a rise in cortisol (Adler, Epel, Castellazzo, & Ickovics, 2000). Because of this, subjective SES is expected to be a stronger predictor of physical health than objective SES, and is the focus of our study.

Examining the interplay between subjective SES and anticipatory coping could be important for implementing new interventions prior to stressful events to reduce physical health symptoms among the older population. Based on previous findings of the mostly beneficial effects of anticipatory coping, we predicted that anticipatory coping efforts would help decrease physical health symptoms. However, because of our dynamic and contextual approach, we expected that not all forms of anticipatory coping would be equally beneficial and some could even be detrimental. We expected that the relationship between anticipatory coping and daily physical health symptoms would depend on subjective SES.

A daily diary micro-longitudinal design was used to assess the relationship between the degree of anticipatory coping each day across participants who rated themselves on a subjective socioeconomic status ladder, and the quantity of physical health symptoms they experienced each day.

> Method

Participants

In 2011, data from 43 adults between the ages of 60-96 years old and across a range of ethnic backgrounds including African American (51.2%), European American (46.5%), and Asian American (2.9%) was collected. Recruitment and compensation included a $10 gift card for the completion of four or fewer days of the study, and a $20 gift card for the completion of five or more days. All participants resided independently within the Greater Raleigh area. A pre-screening for cognitive impairments indicated that the participants were all within the range of normal functioning. Patients scoring an 8 or below on the Short Blessed Test did not meet the cutoff and were excluded from the study (Katzman et al, 1983). The participants reflected diverse educational backgrounds and incomes. Levels of completed education ranged from graduating high school to completing a PhD, MD, or a professional degree. The average education of the participants was some college, and high school graduate was the most frequent among all participants. The minimum annual income ranged within $0-$11,999 and the highest income was $100,000. The mode income category reported was $20,000-$29,999. These factors are important to consider because of their role in influencing how the participants subjectively ranked their own social standing in comparison to the United States.

Procedure

Participants received a questionnaire on the first day of the study to provide baseline data, which included subjective socioeconomic status. The questions answered on this day were not repeated any other day of the study. For the next eight consecutive days, the participants were given a different questionnaire that they completed each day until the conclusion of the study, which provided the data on anticipatory coping and health symptoms. Using a daily diary design improved on methodological limitations from previous studies because it encouraged participants to report on symptoms closer to the time at which they occurred (Stone et al., 1991). The ecological validity of this information was also increased due to the questionnaires being administered to the participants

in their own home.

Measures

Anticipatory Coping

The Measure of Mental Anticipatory Processes (MMAP) is a self-report method directed towards assessing and evaluating both adaptive and maladaptive thought processes that occur when a person considers him or herself anticipating a stressful event (Feldman & Hayes, 2005). We used a modified version of the MMAP (Neupert, Ennis, Ramsey, & Gall, in press) that asked an additional preceding question of, "what is the likelihood you will have an [argument] tomorrow?" followed by the MMAP questions with respect to specific daily stressors (i.e. arguments, disagreements, home stressors, social network stressors, and health stressors) (Almeida, Wethington, & Kessler, 2002). For the purpose of this study, only arguments were considered. Previous work on daily stressful events has shown that interpersonal arguments are among the most frequently occurring stressors for older adults (Neupert, Almeida, Mroczek, & Spiro, 2006)

Plan rehearsal is considered to be an adaptive coping strategy for stressful situations. An example of a question assessing plan rehearsal would be, "I mentally visualize the steps involved in solving the problem". For problem analysis, one may say, "I think about why this problem is happening". A stagnant deliberation item is, "whenever I think about the problem, I often wind up getting stuck", compared to fantasy outcome, "I fantasize about it all just going away". The instructions were adapted to be stressor-specific and targeted the kinds of actions and thoughts that occur in people when they are faced with a potential argument the next day (Neupert et al., in press). Participants were given a Likert scale to use when answering each question: 1 = Never true for me, 2 = Rarely true for me, 3 = Sometimes true for me, 4 = Often true for me, 5 = Always true for me (Feldman & Hayes, 2005). A higher score indicated more of the target construct.

Social Status

Subjective socioeconomic status was based on the participants' self-ranking on a metaphorical ladder which allowed the participants to compare themselves to either the rest of the United States or their community in terms of how much power and how many resources they perceived that they had. We focused our study on the United States comparison. The ladder consisted of ten possible rungs, with socioeconomic status increasing with altitude up the ladder. The top of the ladder represented people who possess the most socioeconomic power, education, and highest income, whereas the bottom rungs represented people of lower power (Adler, 2009). Participants indicated their perceived standing on the ladder by placing an X on the rung that they felt best represented where they stood. The actual rungs where the participants ranked themselves did not cover the entire range of possible rankings (1-10) in comparison to others in the United States. The range occurred from 2-9, while 6 was the most frequently marked rung.

Health Symptoms

Daily physical health symptoms were reported using a version of Larsen and Kasimatis' (1991) physical symptom checklist, which assessed symptoms under the categories of aches and pains, gastrointestinal symptoms, cardiovascular functioning, upper respiratory symptoms, and "other" physical symptoms or discomforts (Larsen & Kasimatis, 1991). The participants were asked to indicate whether or not they experienced each of these symptoms over the last 24 hours. Daily symptoms were calculated each day in terms of the sum of affirmative responses across all of the symptoms.

> Results

Descriptive Statistics

Multilevel models (Raudenbush & Bryk, 2002) that examine nested data; that is, data from days within individuals, were used to address our hypotheses. Anticipatory coping and physical symptom data were measured at the day level (Level 1) and subjective SES was measured at the person level (Level 2). The multilevel models allowed us to examine relationships within and across the two levels.

Significant results were found among all four different coping strategies in regards to arguments, when examined in relation to subjective SES and their effects on physical health symptoms. Two coping strategies, plan rehearsal and stagnant deliberation, yielded significant results for both the United States and community ladders. The other two coping strategies, problem analysis and fantasy outcome, only yielded significant results when looking at SES in terms of comparison to the United States, and did not show significant relationships in comparison to the community. This could possibly be the result of

community being too broadly defined. While all four strategies had significant results, we did not focus our follow-up analyses on problem analysis in this study due to its marginal significance (p = .054), and redundant pattern of agreement across all models.

On average, participants rated themselves on the subjective SES ladder at 5.7. Following previous convention (Cohen, Cohen, Aiken, & West, 2003), the standard deviation (SD = 1.56) was added and subtracted from the average to yield the high and low ladder values for SES in comparison to the U.S. to decompose the interactions. Similarly, the standard deviation of each of the anticipatory coping strategies, plan rehearsal (SD = 1.42), stagnant deliberation (SD = 0.85), and fantasy outcome (SD = 1.11), were added and subtracted from the average values of each of these strategies to find the high and low MMAP values of each one. The mean for plan rehearsal was 2.5 (M = 2.5), for stagnant deliberation (M = 1.8), and for fantasy outcome (M = 1.6). All of these high and low values were used to find the endpoints for graphical lines that described the effect of the corresponding anticipatory coping strategy on physical health with respect to high and low SES groups (see Figures 1-3). We graphed the effects of anticipatory coping for arguments across participants who rated themselves high and low on the socioeconomic status ladder, comparing themselves to the rest of the country, to see how it affected the participants' daily physical health symptoms. Table 1 provides the estimates from the Multilevel Model between plan rehearsal, subjective SES, and physical health outcomes. This model explained 2% of the within person variance and 0% of the between person variance in daily physical health symptoms relevant to the United States. With relevance to the community, this model explained 1% of the within person variance and 0% of the between person variance in daily physical health symptoms. As can be seen in Figure 1, plan rehearsal appeared to be beneficial in reducing physical health symptoms for individuals who envisioned themselves low on the SES ladder. However, for individuals who had high self-ratings, it seemed to be maladaptive and associated with an increase in number of physical symptoms they experienced. When isolating the relationship between plan rehearsal and physical health, we saw a negative relationship. The two negative estimate values for plan rehearsal (-.69 and -.64) indicate inverse relationships for both comparisons to the U.S and the community. As the amount of plan rehearsal increased, the number of health symptoms declined.

Table 2 shows that stagnant deliberation possessed a negative relationship with physical health symptoms. As shown by the negative estimate value (-.82), as stagnant deliberation increased, the number of physical symptoms decreased. This is consistent for both in comparison to the United States, as well as to the community. The model explained 7% of the within person variance and 0% of the between person variance in daily physical health symptoms in the United States. At the community level, the model explained 5% of the within person variance and 0% of the between person variance in daily physical health symptoms. The effects of stagnant deliberation are much higher for participants with a high subjective SES, while participants with low subjective SES experience around the same number of physical symptoms regardless of the degree of stagnant deliberation (see Figure 2).

As seen in Table 3, consistent with plan rehearsal and stagnant deliberation, fantasy outcome was also negatively correlated with physical health symptoms. However, there was only a significant effect for subjective SES in comparison to the U.S., and not the community. The model explained 0% of the within person variance and 0% of the between person variance in daily health symptoms in the United States. Because the model did not explain the within or between person variance, we interpret this significant interaction with caution. Fantasy outcome had a positive effect for both high and low SES participants. Similar to stagnant deliberation, participants with a high subjective SES experience a larger benefit from this anticipatory coping strategy (see Figure 3).

> Discussion

Physical health in older adults was examined along varied amounts of anticipatory coping and levels of subjective SES. We found significant relationships between the effects of anticipatory coping efforts and subjective SES on physical health symptoms in older adults. We found that all four coping strategies were not determinants of physical health alone. However, when observed in combination with subjective SES, our results presented a new idea that high plan rehearsal actually had effects opposite of what we predicted, while high stagnant deliberation and high fantasy outcome supported our hypothesis that coping efforts would be effective in decreasing physical health symptoms across both high and low SES.

A possible explanation for the decrease in physical health in participants with higher subjective SES

could be that having more resources creates more opportunity for worry and rumination. In return, more stressors mean more opportunities to use plan rehearsal, possibly explaining the relationship between these two variables.

Limitations

The findings of the present study should be considered within the context of several limitations. Future research would benefit from a larger sample that includes a larger proportion of men. Although self-reports are often considered a limitation, we note that in daily diary designs like ours, it is important to be able to capture life as it is lived by the participants, so the self-report nature of our data could actually be seen as a strength.

Implications for Future Research

One area of our findings that could be expanded on in future research is the idea of subjective SES. Do individuals perceive their status from where they stand in the community among possible "positions" like volunteering, or retirement, or do they perceive their status from more objective markers like their physical resources? Intergenerational transmission of status could be an important future direction, as parents' completed levels of education (an indicator of objective SES) could have possibly influenced our participants' subjective SES ratings. Looking at parents' education could either confirm or disconfirm an association between the two SES constructs.

> Conclusions

Our results suggest a strong relationship between daily anticipatory coping physical health in older adults which, importantly, is moderated by subjective SES. When considering the predictors of daily physical health in older adults, our study suggests that contextual (i.e., daily anticipatory coping) as well as individual (i.e., subjective SES) characteristics are important to consider, as both tell an interesting piece of the story.

Tables 1 - 3 and Figures 1 - 3 are shown below:

Table 1.

Results for multilevel models examining the relationship between plan rehearsal and participants' subjective SES in relation to the United States.

Fixed Effects	Estimate	Standard Error	p Value
Plan Rehearsal	-.69	.31	.023
Ladder USA	-.53	.21	.019
Plan Rehearsal*LadderUSA	.14	.05	.005

Results for multilevel models examining the relationship between plan rehearsal and participants' subjective SES in relation to the community.

Fixed Effects	Estimate	Standard Error	p Value
Plan Rehearsal	-.64	.38	.09
Ladder Community	-.44	.20	.04
Plan Rehearsal*LadderCommunity	.11	.05	.04

Table 2.

Results for multilevel models examining the relationship between stagnant deliberation and participants' subjective SES in relation to the United States.

Fixed Effects	Estimate	Standard Error	p Value
Stagnant Deliberation	-.82	.42	.06
Ladder USA	-.56	.20	.01
Stagnant Deliberation*LadderUSA	.22	.07	.001

Results for multilevel models examining the relationship between stagnant deliberation and participants' subjective SES in relation to the community.

Fixed Effects	Estimate	Standard Error	p Value
Stagnant Deliberation	-.61	.55	.26
Ladder Community	-.41	.19	.04
Stagnant Deliberation*LadderCommunity	.15	.08	.05

Table 3.

Results for multilevel models examining the relationship between fantasy outcome and participants' subjective SES in relation to the United States.

Fixed Effects	Estimate	Standard Error	p Value
Fantasy outcome	-.35	.32	.28
Ladder USA	-.31	.18	.09
Fantasy Outcome*LadderUSA	.10	.05	.05

Figure 1.

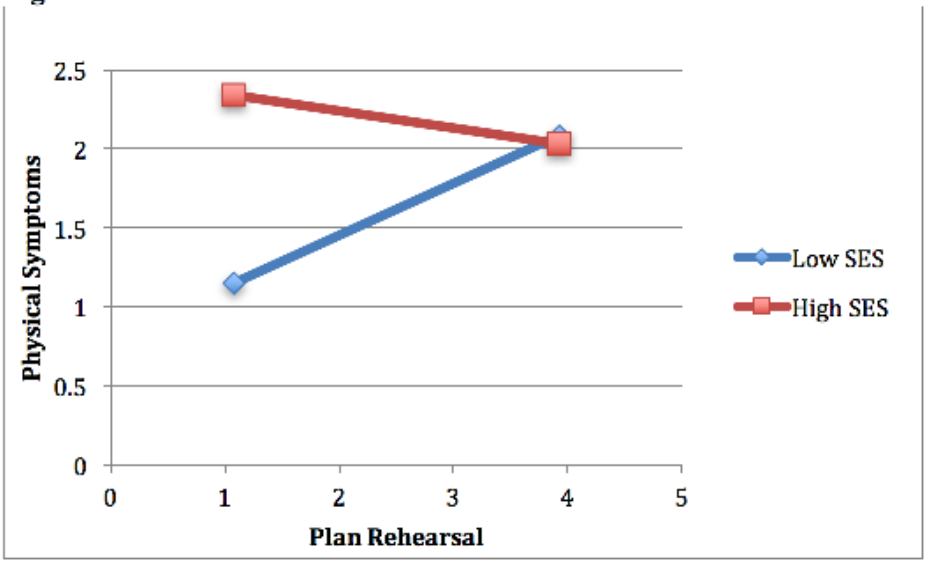


Figure 1. Number of physical health symptoms experienced in older adults in relation to plan rehearsal coping, and depending on high or low SES.

Figure 2.

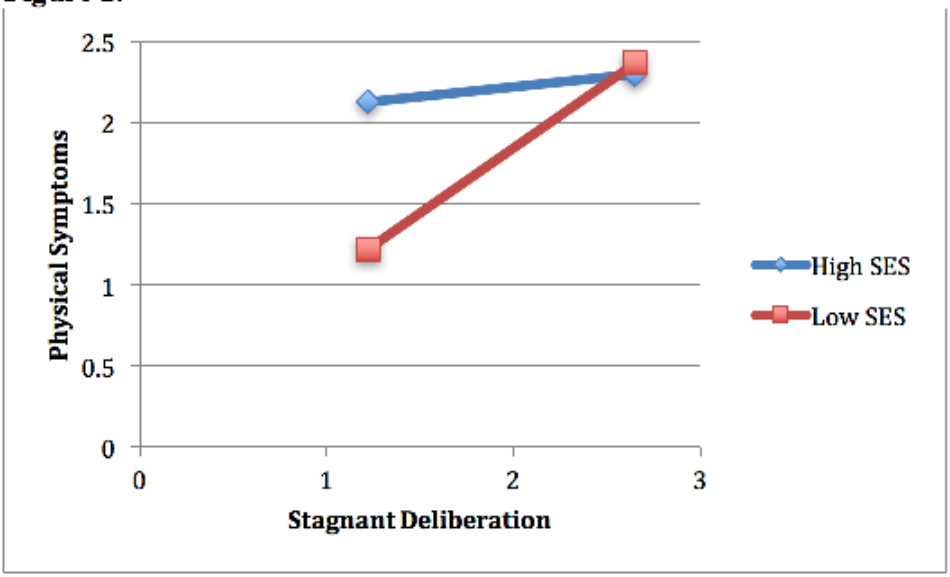


Figure 2. Stagnant deliberation effects on physical health symptoms in older adults depending on high or low SES.

Figure 3.

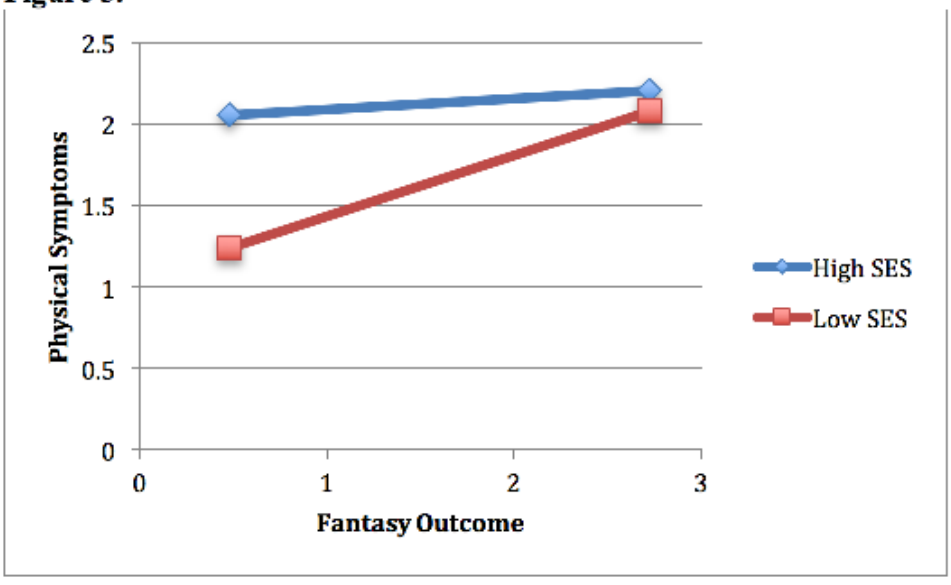


Figure 3. Fantasy outcome effects on physical health symptoms in older adults depending on high or low SES.

> References

Abramson, L. Y., Alloy, L. B., Hankin, B. L., Haeffel, G. J., MacCoon, D. G., & Gibb, B. E. (2002). Cognitive vulnerability-stress models of depression in a self-regulatory and psychobiological context. In I. H. Gotlib & C. L. Hammen (Eds.), Handbook of depression (pp. 268–294). New York: Guilford Press.

Adler, N. E., Epel, E. S., Castellazzo, G. C., & Ickovics, J. R. (2000). Relationship of subjective and objective social status with psychological and physiological functioning: Preliminary data in healthy white women. Health Psychology, 19(6), 586-592.

Almeida, D. M., Wethington, E., & Kessler, R. C. (2002). The daily inventory of stressful experiences (DISE): An interview-based approach for measuring daily stressors. Assessment, 9, 41–55.

Armor, D. A., & Taylor, S. E. (2003). The effects of mindset on behavior: Self-regulation in deliberative and implemental frames of mind. Personality and Social Psychology Bulletin, 29(1), 86–95.

Aspinwall, L. G., & Taylor, S. E. (1997). A stitch in time: Self-regulation and proactive coping. Psychological Bulletin, 121(3), 417–436.

Beck, A. T., Rush, A. J., Shaw, B. F., & Emery, G. (1979). Cognitive therapy of depression. New York: Guilford Press.
Borkovec, T. D. (1994). The nature, functions, and origins of worry. In G. C. L.

Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2003). Applied multiple regression/correlation analysis for the behavioral sciences (3rd ed.). Mahwah, NJ: Erlbaum.

Davey & F. Tallis (Eds.), Worrying: Perspectives on theory, assessment and treatment (pp. 5–33). New York: Wiley.

Davis, R. N., & Nolen-Hoeksema, S. (2000). Cognitive inflexibility among ruminators and nonruminators. Cognitive Therapy and Research, 24(6), 699–711.
D'Zurilla, T. J. & Nezu, A. M. (2001). Problem-solving therapies. In K. S. Dobson (Ed), Handbook of cognitive-behavioral therapies (2nd ed., pp. 211–245). New York: Guilford Press.

Feldman, G., & Hayes, A. (2005). Preparing for problems: A measure of mental anticipatory processes. Journal of Research in Personality, 39, 487-516.

Jacobson, N. S., Dobson, K. S, Truax, P. A., Addis, M. E., Koerner, K., Gollan, J. K., et al. (1996). A component analysis of cognitive-behavioral treatment for depression. Journal of Consulting and Clinical Psychology, 64(2), 295–304.

Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. (1981). Comparison of two modes of stress measurement: Daily hassles and uplifts vs. major life events. Journal of Behavioral Medicine, 4, 1–39.

Katzman R, Brown T, Fuld P, Peck A, Schechter R, Schimmel, H. (1983). Validation of a short orientation-memory concentration test of cognitive impairment. Am J Psyhciatry, 140:734-739.

Larsen, R. J., & Kasimatis, M. (1991). Day-to-day physical symptoms: Individual differences in the occurrence, duration, and emotional concomitants of minor daily illnesses. Journal of Personality. Special Issue: Personality and Daily Experience, 59, 387–423.

Lazarus, R. S., & Folkman, S. (1984). Stress, appraisal, and coping. New York: Springer.

Linehan, M. M. (1993). Cognitive-behavioral treatment of borderline personality disorder. New York: Guilford Press.

Lowenthal, M. F., Thurnher, M., & Chiriboga, D. A. (1975). Four stages of life: A comparative study of men and women facing transitions. San Francisco: Josey Bass.

Mallers, M. H., Almeida, D. M., & Neupert, S. D. (2005). Women's daily physical health symptoms and stressful experiences across adulthood. Psychology and Health, 20(3), 389-403.

Marlatt, G. A., & Gordon, J. R. (Eds.). (1985). Relapse prevention: Maintenance strategies in the treatment of addictive behaviors. New York: Guilford Press.
Mor, N., & Winquist, J. (2002). Self-focused attention and negative affect: A meta-analysis. Psychological-Bulletin, 128(4), 638–662.

Neupert, S. D., Almeida, D. M., Mroczek, D. K.,& Spiro, A. (2006). Daily stressors and memory failures in a naturalistic setting: Findings from the VA Normative Aging Study. Psychology and Aging, 21, 424-429.

Neupert, S.D. Ennis, G.E., Ramsey, J.L., & Gall, A.A. (in press). Solving Tomorrow's Problems Today? Daily Anticipatory Coping and Reactivity to Daily Stressors. Journal of Gerontology: Psychological Sciences.

Nolen-Hoeksema, S. (2000). The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. Journal of Abnormal Psychology, 109(3), 504–511.

Oettingen, G. (1996). Positive fantasy and motivation. In P. M. Gollwitzer & J. A. Bargh (Eds.), The psychology of action: Linking cognition and motivation to behavior (pp. 236–259). New York: Guilford Press.

Pyszczynski, T., & Greenberg, J. (1987). Self-regulatory perseveration and the depressive self-focusing style: A self-awareness theory of reactive depression. Psychological Bulletin, 102(1), 122–138.

Raudenbush, S.W. and Bryk, A.S. (2002). Hierarchical Linear Models (Second Edition). Thousand Oaks: Sage Publications.

Rossi, A. S. (1980). Life-span theories and women's lives. Signs, 6, 4–32.

Schwarzer, R., & Knoll, N. (2003). Positive coping: Mastering demands and searching for meaning. In S. J. Lopez & C. R. Snyder (Eds.), Positive psychological assessment: A handbook of models and measures (pp. 393–409). Washington, DC: American Psychological Association.

Segerstrom, S. C., Stanton, A. L., Alden, L. E., & Shortridge, B. E. (2003). A multidimensional structure for repetitive thought: What's on your mind, and how, and how much? Journal of Personality and Social Psychology, 85(5), 909–921.

Sherman, R. T., & Anderson, C. A. (1987). Decreasing premature termination from psychotherapy. Journal of Social and Clinical Psychology, 5(3), 298–312.

Stiles, W. B., Elliott, R., Llewelyn, S. P., Firth-Cozens, J. A., Margison, F. R., Shapiro, D. A., et al. (1990). Assimilation of problematic experiences by clients in psychotherapy. Psychotherapy: Theory, Research, Practice, and Training, 27(3), 411–420.
S
tone, A. A., Kessler, R. C., & Haythornthwaite, J. A. (1991). Measuring daily events and experiences: Decisions for the researcher. Journal of Personality, 59, 575 607.

Suinn, R. M. (1997). Mental practice in sport psychology: Where have we been, where do we go?. Clinical Psychology: Science and Practice, 4(3), 189–207.

Taylor, S. E. (1983). Adjustment to threatening events: A theory of cognitive adaptation. American Psychologist, 38(11), 1161–1173.

Taylor, S. E., & Schneider, S. K. (1989). Coping and the simulation of events. Social Cognition, 7(2), 174–194.

Taylor, S. E., Pham, L. B., Rivkin, I. D., & Armor, D. A. (1998). Harnessing the imagination: Mental stimulation, self-regulation, and coping. American Psychologist, 53(4), 429–439.

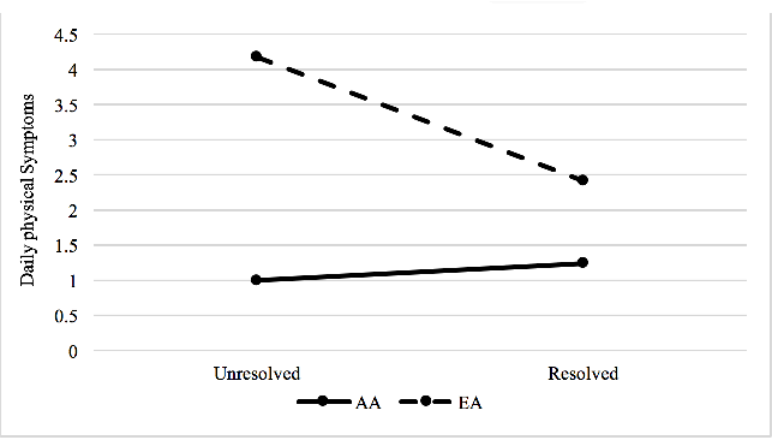
Verbrugge, L. M. (1986). From sneezes to adieux: Stages of health for American men and women. Social Science Medicine, 22, 1195–1212.

Verbrugge, L. M. (1989). The twain meet: Empirical explanations of sex differences in health and mortality.

DOES ETHNICITY AFFECT HOW OLDER ADULTS DEAL WITH STRESSORS AT HOME?

by **Sara S. Monazah, Jennifer A. Bellingtier, Ph.D., Agnes A. Gall, M.S. & Shevaun D. Neupert, Ph.D.**

Mentors: **Shevaun D. Neupert, Ph.D., Jennifer A. Bellingtier, Ph.D. & Agnes A. Gall, M.S.**



Ethnicity differences (AA: African American; EA: European American) in physical reactivity to home stressor resolution. Reactivity is operationalized as the within-person association between home stressor resolution and daily physical symptoms.

Sara S. Monazah is a recent graduate of NC State University where she earned her B.A. in Psychology. She has studied the aging population with a specific interest in how ethnicity can affect home stressor resolution. This fall Sara will begin her Master's Degree in the department of Social Work at NC State University.

Jennifer A. Bellingtier, Ph.D. is a recent graduate of NC State University's doctoral program in lifespan developmental psychology. Her research interests focus on individual's attitudes, beliefs, and feelings about their own aging, as well as how those beliefs develop and impact well-being. In the fall she will begin work as a post-doctoral research associate at Friedrich Schiller University in Jena, Germany.

Agnes A. Gall, M.S. received her master's degree in Lifespan Developmental Psychology from NC State University. She is interested in the relationships between perceptions of control and coping as they change within a person over time.

Shevaun D. Neupert, Ph.D. is an Associate Professor of Lifespan Developmental Psychology at NC State University. Her research focuses on the dynamic interplay of individual differences and contextual changes associated with health and well-being on a daily basis. She seeks to examine individual and daily characteristics that address the following question: For whom and under which

>Abstract

Numerous studies have examined stress in older adults but little research has examined the specific relationship between ethnicity and home stressors. We used a micro-longitudinal, eight-day daily diary study to examine reactivity to home stressors in older adults. 42 participants from the Greater Raleigh Area documented via these diaries for 337 days. Each participant's age ranged from 60 to 96 and participants were categorized into either European-American or African-American. Participants reported their daily home stressor resolution (resolved or unresolved) and daily physical symptoms (e.g., joint pain, fatigue, headache). We found that European-Americans had a significantly higher number of physical symptoms on days with an unresolved home stressor than that of their African-American counterparts. Findings suggest that there are ethnic differences in the physical effects of home stressor resolutions among older adults. Awareness of disparity is an important first step in closing this health gap and ethnicity should be included in future assessments of home stressors in older adults.

> Introduction

Everyone experiences stress, and the older adult population is no exception (Almeida & Horn, 2004). While numerous studies have examined stress in older adults (Seeman & Robbins, 1994; Neupert, Almeida, & Charles, 2007), many studies focus mainly on broad conceptualizations of stress. There is a need for research that examines the nuances of reactions to specific stressors (Koffer, Ram, Conroy, & Almeida, 2016). Little research has been done on the relationship between ethnicity and home stressors in older adults. Home stressors refer to any stressors that occur in the household (e.g., household maintenance, neighborhood concerns, and financial problems). As one of the primary locations where older adults spend their time, it is important to examine the implications of home stressors. Almeida (2005) stated that there are sociodemographic factors that may contribute to how an individual deals with day-to-day stress including age, gender, education, income, marital status and parental status. Although ethnicity was not originally considered to be a sociodemographic factor, it may be relevant to how reactive individuals are to home stressors. For example, Wadsworth and Santiago (2008) found that, unlike European Americans, African Americans show no link between several psychological syndromes and family trouble. Research is increasingly considering what effect an individual's ethnicity may have on how they handle stress (Cichy, Stawski, & Almeida, 2012).

An unresolved home stressor occurs when an individual continues to experience stress surrounding their home life because the stressor has not been dealt with or resolved on its own. A stressor is considered resolved when an individual has figured out a way to deal with the stressor or the stressor disappears by itself. Successfully resolving a home stressor gives older adults experience in successfully managing stress and may allow older adults to become even better at coping (Brennan, Schutte, & Moos, 2006).

Home stressors deal with the responsibilities that occur in the home setting; this can include dealing with family issues, typical homeowner problems, and interpersonal conflicts that take place in the home (Hay & Diehl, 2010). Home stressors may not be the predominant source of stress for older adults (Almeida & Horn, 2004); however, due to the amount of time older adults spend at home it is important to understand their impact. In older adulthood, the close family network becomes more emotionally important and salient as other social relationships may be decreasing, therefore this may be why it remains a source of stress in adulthood (Carstensen, 1992). Home stressors can also include a wide range of other things, including: pet problems, financial problems, scheduling conflicts, having too much to do, neighborhood concerns, and household maintenance (Almeida & Horn, 2004).

Stress-related increases in physical symptoms are one way stress can be effectively measured (Almeida, Wethington, & Kessler, 2002; Neupert et al., 2007). Neupert et al. assessed age differences in young, middle and older adults and found no differences in age on physical reactivity when looking specifically at home stressors. Ethnic differences were not included in their analyses however, so the current study aims to merge important person-level (i.e., ethnicity) and contextual-level (i.e., home stressors) information to further explore physical reactivity to stressors.

Cichy et al. (2012) looked at differences in ethnicity as they pertain to daily family stressors. Family stressors are different than home stressors in that they are focused on the daily struggles of family life and how these struggles impact relationships in the family regardless of where they occur (Almeida, 2005; Cichy et al.). Home stressors focus on stresses that occur solely within the home. Similar to this present study, two ethnicities were represented: European Americans and African Americans. Cichy et al. found European Americans and African Americans were similar in emotional reactivity; however, African Americans were more physically reactive to stressors in that the reactivity continued to impact the individual the day after the stressor occurred.

Research has shown that individuals with a lower socioeconomic status (SES) are likely to have more chronic stress than individuals who have a higher status (Turner et al., 1995). It is also assumed that due to more chronic stress, individuals of a lesser status will report more stressful events than those of the higher status (Turner et al., 1995). Grzywacz, Almeida, Neupert, and Ettner (2004) found that the more education an individual had, the more stressors the individual reported. Although higher education was associated with more stressors, people with less education had a more severe response to stressors whenever a stressor did occur (Grzywacz et al, 2004). Given that ethnic minorities tend to have lower SES than ethnic majorities in the US (Crimmins, Hayward, & Seeman, 2004), an important question for the current study is whether the SES differences found in previous studies are similar to or different from ethnic differences in the stress process. A

strength of the current study is that there were no significant differences in income across ethnicity.

We are unaware of any studies that have looked at the possible role that an individual's ethnicity may have when assessing home stressors and physical symptoms. Although one study explored race/ethnicity differences as it pertains to family stressors and did not find any differences in emotional reactivity (Cichy et al., 2012) we were interested in focusing on two ethnic groups (European Americans and African Americans) and the potential association between unresolved home stressors and daily physical health. Older adults are likely to feel the physical wear of everyday home stressors, and as Almeida (2005) pointed out, some individuals may be in a better position to deal with the stressors than others. It may be that an individual's ethnicity is one sociodemographic factor that contributes to how reactive the individual is to the home stressor, especially in regards to a greater risk for physical symptoms.

Present Study

A micro-longitudinal, eight day-daily diary study was conducted to address whether the ethnicity of older adults (aged 60-96) is associated with more or less physical symptoms in response to resolved and unresolved home stressors. There are many benefits to using the daily diary method (Almeida, 2005). Daily stressors are crucial to look at because they are the everyday worries and troubles that plague an individual's life. The daily diary method allows for an in-depth understanding of an individual's everyday troubles and worries (Almeida, 2005). Since participants are able to report daily occurrences in a more detailed fashioned as they occur rather than having to think back on past experiences (Bolger, Davis, & Rafaeli, 2003) the method has more external validity (Almeida, 2005; Bolger, Davis, & Rafaeli, 2003). Similar to previous SES findings, we hypothesize that European Americans will have more stressors than African Americans. However, we also hypothesize that when unresolved home stressors occur, African Americans will be more reactive.

> Methods

Participants & Procedures

Participants were part of a larger study, the Anticipatory Coping Every Day study (ACED, Neupert, Ennis, Ramsey, & Gall, 2016), that occurred in 2011 in the Greater Raleigh Area. In the current analyses, 42 adults aged 60-96 years old, with a mean age of 74.74 years, were assessed. African Americans represented 52.4% (n=22) of the participants while European Americans represented 47.6% (n=20) of the participants. In order to be eligible to participate, participants were screened for cognitive impairments which was done using the Short Blessed Test (Katzman et al., 1983). Any participant that received an eight or lower on the test was deemed cognitively able to participate. The participants completed a demographics questionnaire on Day 1 as well as other information that became referred to as the baseline data. This information included the participant's age, ethnicity, education, etc. Education ranged from some high school to graduate degrees. The mode education was a high school degree, while the average education was some college. There were no ethnic differences in education. Income was evaluated at $10,000 intervals with the lower range being $0-$19,999 and the highest $100,000 or more. The median range was from $20,000 to $29,999. There were no significant difference in income across ethnicity. Following the initial baseline data, the participants completed eight (consecutive) daily diaries. The participants filled out the diaries in the comfort and privacy of their own homes lending to the external validity of the study. Compensation for time was also given; participants that completed five or more days received a $20 gift card and participants that completed four or fewer days received a $10 gift card.

> Measures

Home Stressors

Home stressors were evaluated using the Daily Inventory of Stressful Events (DISE; Almeida et al., 2002). The participants were asked if, in the past 24 hours, anything occurred at home that most people would consider to be stressful. If the participant marked no, no further questions were asked. However, if they marked yes, they indicated who was involved, what was the main source of the stressor, and if the issue was resolved or unresolved. Home stressors included: pet problems, financial problems, scheduling conflicts, having too much to do, neighborhood concerns, and household maintenance. Only data from days where people marked yes to having a home stressor were included in the study.

Physical Symptoms

Each participant completed a physical symptoms inventory which was taken from the Larsen and Kasimatis' (1991) physical symptom checklist. The participants were asked if, in the past 24 hours, they

had experienced any of the physical symptoms that were listed and if so to check all that applied for that day. Examples of symptoms included: headache, backache, chest pains, shortness of breath or difficulty breathing, etc. A daily composite was created by summing the number of affirmative responses.

> Results

Home stressors occurred on 12.50% of days, for a total of 42 instances. Frequency distributions showed the other people involved included: no one 28.6% (n=12), spouse 26.2% (n=11), grandchild 16.7% (n=7), child 11.9% (n=5), another family member 4.8% (n=2), friend 2.4% (n=1), neighbor 2.4% (n=1), and other person not specified 7.2% (n=3). Frequency distributions for main topics of the stressors revealed that the main topic of the stressor were: health 21.4% (n=9), schedule issues 16.7% (n=7), household maintenance 16.7% (n=7), having too much to do 11.9% (n=5), pet related issues 4.8% (n=2), finance 2.4% (n=1), and other topic not specified 26.4% (n=11). In regards to resolution, 75% of the home stressors were resolved leaving 25% unresolved. Consistent with our hypothesis, results of an independent t-test showed that European Americans (M = 0.17, SD = 0.37) had more daily home stressors than African Americans (M = 0.09, SD = 0.29): t = -1.98, p = .049, d = -0.21, r = 0.10.

We used a multilevel model (Raudenbush & Bryk, 2002) to predict the number of physical symptoms on a daily basis as a result of home stressors and whether those stressors were resolved or unresolved. Multilevel modeling was chosen because it examines both between-person differences (i.e., ethnicity) and the daily within-person fluctuations (i.e., stressors and physical symptoms).

First, an unconditional model was ran in order to assess whether there was significance between-person (Level 2) and within-person (Level 1) in the variance of daily physical symptoms. Significant variance was found at both the between person (τ_{00} = 1.87 (0.46), t=4.08, p < .001) and within person (σ^2 = 1.40 (0.12), t=11.77, p <.001) level. Results from the intraclass correlation coefficient (Raudenbush & Bryk, 2002) revealed that 57% of the variance in daily physical health symptoms was at the between-person level and 43% was at the within-person level. These results indicate that there is sufficient variance at both levels to continue with subsequent analyses. To address our hypotheses, the following multilevel model was tested:

Level 1: Physical Symptoms $_{it}$

$$= \beta_{0it} + \beta_{1it} \text{ (Stressor Resolution)} + r_{it}$$

Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}$ (Ethnicity) + u_{0i}

$$\beta_1 = \gamma_{10} + \gamma_{11} \text{ (Ethnicity)} + u_{1i}$$

There was a significant main effect of ethnicity, γ_{01} =3.03 (0.69), t=4.41 p=.001, with European Americans reporting more physical symptoms than African Americans. This main effect was qualified by a significant interaction between ethnicity and resolution of home stressor, γ_{11} = -1.80 (0.86), t=-2.08, p=.04 (see Figure 1). European Americans had significantly more physical symptoms than African Americans when the home stressor was unresolved. This model explained 58% of the between-person variance and 6% of the within-person variance in daily physical symptoms.

The interaction was decomposed by conducting separate models for each ethnic group. The slope for European American participants was significant, slope = -1.33 (0.65), t=-2.05, p=.05, meaning that European Americans experienced a significant decrease in physical symptoms when home stressors were resolved whereas African Americans reported a similar low level of physical symptoms when home stressors were resolved or unresolved. The test of contrast for unresolved stressors was significant, difference = 2.97 (0.93), t=3.19 p=.004, suggesting that European Americans had more physical symptoms when home stressors were unresolved than African Americans.

A chi-squared test was performed in reference to the follow-up questions that were asked of each participant upon them marking "yes" to having had a stressor occur. The participants indicated who was involved in the stressor and what the main topic of the stressor was. The chi-squared test did not reveal any significant differences in stressor resolution by the person involved in the stressor or by main topic of stressor, except for health-related stressors, χ^2 (1) = 6.07, p =. 01. A negative correlation was found between stressor resolution and health topic showing that home stressor involving a health problem were less likely to be resolved. However there was not a significant correlation or chi-square for having a health-related stressor in the home and number of physical symptoms on a particular day. Because of the significant effects for stressor resolution, total daily physical health symptoms were added into the multilevel model as a control, but did not impact the results or contribute unique significant variance; thus,

for the model we present this variable was removed.

> Discussion

This study has contributed to the understanding of ethnicity, daily home stressors, and daily physical health symptoms. We evaluated home stressor resolution (unresolved or resolved) in terms of two ethnic groups, European Americans and African Americans, and evaluated differences in reactivity in terms of physical symptoms. Our findings suggested a significant difference in reactivity by ethnicity. The experience of home stressors may differ by ethnic group. Our findings partially supported our original hypothesis since European Americans did end up having more home stressors than did African Americans; however, it was European Americans, not African Americans, who tended to be more reactive to unresolved home stressors, resulting in more daily physical symptoms.

Almeida (2005) noted that daily diary studies can look at sociodemographic information like age, gender, and marital status; factors that could create differences in how the individual deals with stress. Cichy et al. (2012) included race/ethnicity as a sociodemographic factor in their study and found that African Americans were more physically reactive a day after the stressor had occurred than European Americans, but found no ethnic differences in emotional reactivity to the family stressor (Cichy et al.). It is important to assess how ethnicity may impact the appraisal of stressors in different ways. The present study emphasizes the idea that ethnicity should continue to be a sociodemographic factor that is considered because it may impact how an individual handles home stressors.

One possibility for the difference between European American and African Americans when it comes to unresolved home stressors may have to do with culture. There could be some fundamental differences in how stress is handled amongst ethnicities based on their culture and values (Kuo, 2011). The two ethnic groups may react to home stressors differently based on how important they view the problem causing the stressor to be. The focus of the daily diary method is to bring attention to the everyday struggles and stresses of an individual's life (Almeida, 2005); this study focused specifically on those stressors that occurred in the context of the home. Future research is necessary to try to understand whether cultural differences may play a role in the importance or perception of home stressors.

This paper found that European Americans have more physical symptoms when a home stressor is unresolved than African Americans. This information has the potential to help older adults living in the United States to better understand themselves and how potential stress may affect them. European Americans and African Americans can benefit from understanding how their ethnicity may impact the physical symptoms they endure due to unresolved home stressors. If older adults realize that unresolved home stressors can have an influence on how many physical symptoms they have, then they may try to resolve the unresolved issue causing them stress. Older adults can benefit from resolving a stressor because it may result in even better future coping (Brennan, Schutte, & Moos, 2006).

Limitations and Future Directions

While this study has contributed to the current understanding of ethnicity and home stressor reactivity in the older adult population there were some limitations. This study was limited in only being able to compare home stressor reactivity with two ethnic groups. It would be interesting to see how other groups of people compare with each other. This research study also had 93% female participants. Gender differences have been found for other stressors (Almeida, 2005). Almeida found that men had more work and school stressors and women had more network stressors. Future research should continue to look at ethnicity as a sociodemographic factor that can affect how an individual reacts to home stressors, as well as exploring whether it affects other sorts of stressors like network and work stressors. Future researchers can also take a look at gender differences across ethnicities to explore whether men and women within ethnic groups experience similarities or differences in stress reactivity. Researchers can continue to explore ethnic differences between European Americans and African Americans in other age groups. The field can move forward by exploring the impact and importance that ethnicity may have on understanding how each individual deals with stressors.

Conclusion

This study set out to explore ethnicity differences in home stressors among older adults. We found that European Americans tended to be more reactive than African Americans to unresolved home stressors which leads to them experiencing more physical symptoms on a day-to-day basis. We have shown that ethnicity is an important sociodemographic factor

that should be taken into account when assessing the individual differences in older adults' reactivity to daily home stressors.

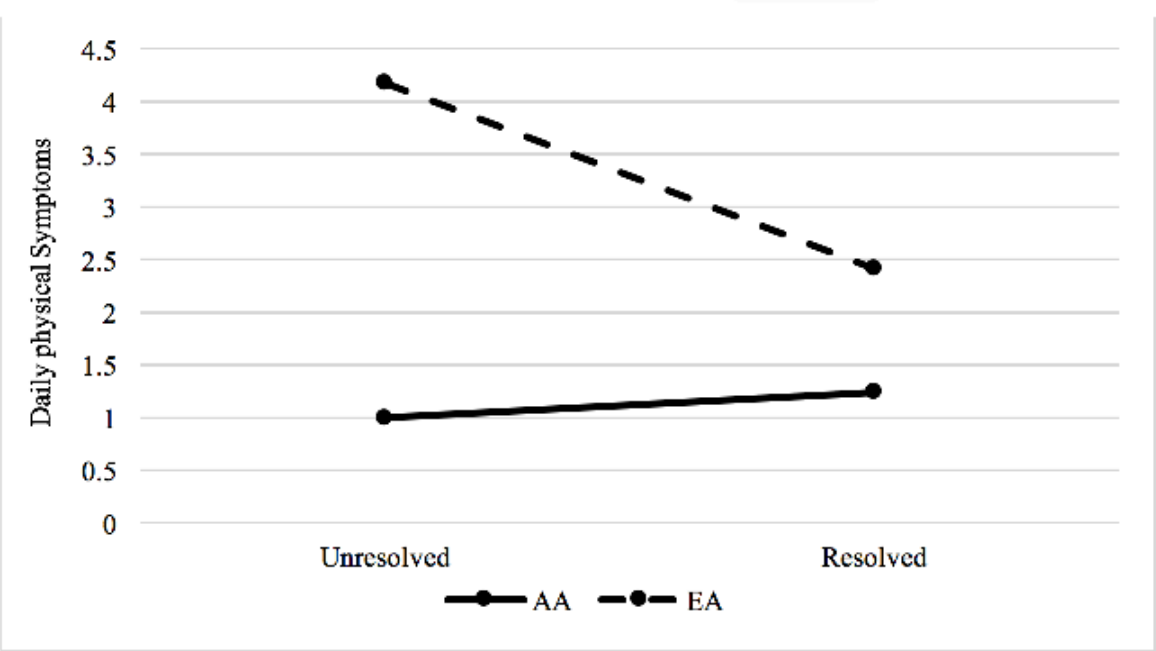


Figure 1: Ethnic differences (AA: African American; EA: European American) in physical reactivity to home stressor resolution. Reactivity is operationalized as the within-person association between home stressor resolution and daily physical symptoms.

> References

Almeida, D. M. (2005). Resilience and Vulnerability to Daily Stressors Assessed via Diary Methods. *Current Directions in Psychological Science*, 14(2), 64-68. doi:10.1111/j.0963-7214.2005.00336.x

Almeida, D.M., & Horn, M.C (2004). Is Daily Life More Stressful during Middle Adulthood? In O.G Brim, C.D Ryff, R.C Kessler (Eds.). *How healthy are we?: A national study of well-being at midlife* (p. 425-451). Chicago, IL, US: University of Chicago Press.

Almeida, D. M., Wethington, E., & Kessler, R. C. (2002). The Daily Inventory of Stressful Events (DISE): An interview-based approach for measuring daily stressors. *Assessments, 9*(1), 41-55. doi:10.1177/1073191102009001006

Bolger, N., Davis, A., & Rafaeli, E. (2003). Diary methods: Capturing life as it is lived. *Annual Review of Psychology. 54*579-616. doi:10.1146/annurev.psych.54.101601.145030

Brennan, P.L., Schutte, K.K., & Moos, R.H. (2006). Long-term patterns and predictors of successful stressor resolution in later life. *International Journal of Stress Management*, *13*(3), 253-272. doi:10.1037/1072-5245.13.3.253

Carstensen, L.L. (1992). Social and emotional patterns in adulthood: Support for socioemotional selectivity theory. *Psychology And Aging*, *7*(3), 331-338. doi:10.1037/0882-7974.7.3.331

Cichy, K.E., Stawski, R. S., & Almeida, D. M. (2012). Racial differences in exposure and reactivity to daily family stressors. *Journal of Marriage and Family, 74*(3), 572-586.

Crimmins, E. M., Hayward, M. D., & Seeman, T. E. (2004). Race/Ethnicity, Socioeconomic Status, and Health. In N. B. Anderson, R. A. Bulatao, & B. Cohen (Eds.), *Critical Perspectives on Racial and Ethnic Differences in Health in Late Life* (pp. 310-352). Washington, DC: The National Academies Press.

Grzywacz, J. G., Almeida, D. M., Neupert, S. D., & Ettner, S. L. (2004). Socioeconomic status and health: A micro-level analysis of exposure and vulnerability to daily stressors. *Journal of Health and Social Behavior, 45*(1), 1-16.

doi:10.1177/002214650404500101

Hay, E. L., & Diehl, M. (2010). Reactivity to daily stressors in adulthood: The importance of stressor type in characterizing risk factors. *Psychology and Aging, 25*(1), 118-131. doi:10.1037/a0018747

Katzman, R., Brown, T., Fuld, P., Peck, A., Schechter, R., & Schimmel, H. (1983). Validation of a Short Orientation-Memory-Concentration Test of Cognitive Impairment. *American Journal of Psychiatry, 140,*734–739.

Koffer, R.E., Ram, N., Conroy, D.E., Pincus, A.L., & Almeida, D.M. (2016). Stressor diversity: Introduction and empirical integration into the daily stress model. *Psychology and Aging, 31*(4), 301-320. doi:10.1037/pag000009

Kuo, B.H. (2011). Culture's consequences on coping: Theories, evidence, and dimensionalities. *Journal Of Cross-Cultural Psychology*, 42(6), 1084-1100. doi:10.1177/0022022110381126

Larsen, R.J., & Kasimatis, M. (1991). Day-to-day physical symptoms: Individual differences in the occurrence, duration, and emotional concomitants of minor daily illnesses. *Journal of Personality, 59*, 387-423.

Neupert, S.D., Almeida, D. M., & Charles, S. T. (2007). Age differences in reactivity to daily stressors: The role of personal control. *The Journal of Gerontology: Series B: Psychological Sciences and Social Sciences, 62B(4),* 216-P225. doi:10.1093/geronb/62.4.P216

Neupert, S.D., Ennis, G.E., Ramsey, J.L., & Gall, A.A (2016). Solving Tomorrow's Problems Today? Daily Anticipatory Coping and Reactivity to Daily Stressors. *Journal of Gerontology: Psychological Sciences 71*(4), 650-660. doi:10.1093/gernob/gbv003

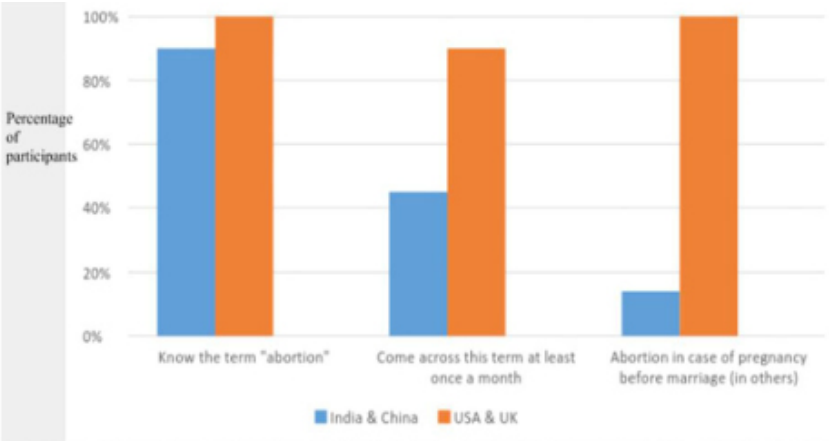
Raudenbush, S. W., & Bryk, A. S. (2002). *Hierarchical linear models: Applications and data analysis methods* (2nd ed.). Newbury Park, CA: Sage.

Seeman, T.E., & Robbins, R.J. (1994). Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrinology Review, 15*, 233-600.

Turner, R.J., Wheaton, B., & Lloyd, D.A (1995). The epidemiology of social stress. *American Sociological Review, 60*(1), 104-125. doi:10.2307/2096348

Wadsworth, M.E., & Santiago, C.D. (2008). Risk and resiliency processes in ethnically diverse families in poverty. *Journal of Family Psychology, 22*(3), 399-410. doi:10.1037/0893-3200.22.3.399

INFLUENCE OF POWER DISTANCE ON ABORTION STUDIES



Graph representing result-abortion attitude in high power distance countries (Group A: India & China) and low power distance countries (Group B: United States of America & United Kingdom).

by **Jeen Shaji**
Mentor: **Bethany Bradshaw**

Jeen Shaji is a Junior pursuing a B.S. in Economics at NC State University. Jeen also loves dancing and is on Thandav at NC State, an Indian classical dance team. Jeen has been interested in research since freshman year and hopes to pursue a Ph.D. in the future.

> Abstract

Many scholars have discussed about varying abortion attitudes in different countries, but little is known about factors influencing this irregularity from one country to another. Bahr and Marcos (2003), Wilson et al. (2011) and Scheepers et al. (2002) consider religious affiliation and education as factors affecting moral liberalization, which influences abortion attitudes. A notable deviation with most studies is that the effect of education doesn't hold true across different nationalities. However, none of them investigate the reason behind such crossborder differences. In response, my research aims to fill this "gap" by studying the relation between power distance practiced in a country and abortion attitudes amongst students of similar educational levels. Data was collected from college students from India and China (countries practicing high power distance), and from the USA and the UK (countries practicing low power distance) using an online survey. Amongst other results, it was found that 17% students from the US and the UK knew 5 to 10 people who had considered abortion compared to 0% from India and China. All students (100%) from the US and the UK chose pregnancy before marriage as a reason for abortion amongst known people, compared to 17% from India and China. This can be an indicator of relatively more liberal abortion attitudes in countries practicing low power distance. These results may indicate that transition of a country from high power to low power distance, a consequence of westernization, can possibly lead to more liberal abortion attitudes.

> Introduction

In this modern and sophisticated world, literacy has evolved from being merely the ability to read and write to an inseparable aspect of human nature. This multicultural and competitive world has developed literacy into one of the key factors influencing our career and social life. The term literacy has been attributed to various definitions with time, but as Scribner (1984) states, no single definition can capture its "essence" (p. 7). The increasing literacy rate in most countries over the last two decades is an indicator of the growing importance of education.

Several scholars have observed that there is a significant difference in how educated and uneducated people respond to similar situations. This can be linked to changes in human perception with education. According to most scholarly articles, education has led to liberalization in moral attitudes. Scheepers, Grotenhuis & Van Der Slik (2002) affirm this relation in their study, by considering acceptability of abortion, homosexuality and premarital relations (p. 162). A notable deviation with their study is that, this effect of education does not hold true across different nationalities. In another research study, Bahr & Marcos (2003) conclude that education has a lesser impact on sexual liberalism and abortion attitudes in Greece than in the United States of America (p. 421). This crossnational difference indicates that education cannot be solely treated as an indicator of liberalization.

Even though several scholarly articles have discussed abortion attitudes in various countries, the reason for such differences are not explained. Hence, my research aims to fill this "gap" by studying the effects of power distance culture practiced in a country on abortion attitudes amongst students of similar educational level. Power distance can be explained as the degree to which people are willing to accept the unequal distribution of power. A country is said to practice high power distance if it accepts unequal distribution of power and vice versa (Rutledge, 2011). For example, obedience and respect are prominent teachings of societies that practice high power distance while low power distance culture revolves around equality. This is important because youth of developing high power distance countries like India and China, are being attracted by the western low power distance culture. My hypothesis is that education would lead to a more liberal view in countries that practice low power distance than those that practice high power distance. Thus, the transition of a high power distance country into a low power distance will lead to a rise in rate of abortion given that my hypothesis is justifiable.

> Literature Review

In an earlier research study, Scheepers et al. (2002) hypothesizes that, "the longer individuals have been exposed to the education system, the less likely they are to subscribe to conservative moral attitudes" (p. 160). This claim was supported by results indicating that more pious and less literate people supported conservative moral values. Wilson, Garcia, Olavarrieta, VillalobosHernández, Rodríguez, Smith, & Burks (2011) claim education to be the "most consistent predictor" for support and legalization of abortion in 2008 and 2009, the years after the landmark reform[1] was introduced in Mexico (p. 180). Further, the data collected by Bahr & Marcos (2003) is consistent with this outcome as it gives importance to sexual liberalism as an intermediate stage towards abortion, which is influenced by higher level of education (p. 408). In other words, these articles discuss moral liberalization as an outcome of increasing literacy. Conversely according to Wang (2014), educated women are less likely to undergo induced abortion. Here, higher education is linked with efficient use of other contraceptive methods. The above stated studies have indicated a positive relation between education and liberalization of moral attitudes. However, it is less clear whether certain other physical, social and contextual factors have an impact on this.

Most scholars agree that religious practices play an essential role in the development and demonstration of an individual's attitude. Wilson et al. (2011) and Scheepers et al. (2002) consider religious affiliation and practices as an indicator of moral liberalization in their study. In Mexico, higher acceptance of the reform is attributed to the increasing awareness, knowledge of the law and decreasing piousness (Wilson et al., 2011, p. 180). In most cases, the data collected by Wilson et al. (2011) is consistent with that of Scheepers et al. (2002) which shows that frequent participation and involvement in church activities by parents led to conservative moral attitudes amongst children. We cannot quantitatively measure values induced by one's religion due to which some people may challenge the view these scholars propose. However, regularity and devotion towards religious practices become a part of our social life, thus inducing certain behaviors. Even though these scholarly articles tell a great deal about factors influencing attitudes towards

1. On 24th April 2007, the Mexico City Legislative Assembly legalized elective firsttrimester abortion.

abortion, they have not been able to explain cross-national differences.

In their study, Bahr & Marcos (2003) record that education has a mean value of 2.51 in Greece and 2.18 in the United States of America on a 3 point scale (p. 414), leading to the conclusion that education has a lesser impact on sexual liberalism and abortion attitudes in Greece, than in the United States of America. Scheepers et al. (2002) agrees with this observation as a notable deviation from the result of his study that this effect of education and religion does not hold true across different nationalities. Consequently, it is necessary to find a reason for this varying trend across borders. Hence, as stated in the introduction, I will explore this gap in my research study by concentrating on how power distance practiced in a country effects abortion attitudes.

> Methods

In this study, I surveyed students from countries that practicing high power distance or low power distance. Most Asian countries like India and China practice high power distance whereas the United States of America and the United Kingdom practices low power distance ("Power Distance Index," n.d.). *Table 1* represents the data used to support the above statement.

	Country	Power Distance Index	Practiced Power Distance
Group A	China	80	High
	India	77	High
Group B	United States of America	40	Low
	United Kingdom	35	Low

Table 1: Power distance practiced in participating countries ("Power Distance Index," n.d.).

The sample was divided into two groups for convenience. Group A included students from India and China, countries that practice high power distance and Group B consisted of students from the United States of America and the United Kingdom, countries that practice low power distance.

A questionnaire was sent to students who had spent a major part of their life, especially high school or college in any one of these countries. The survey was made using "Qualtrics", one of the most efficient online tools for collecting and comparing data. The survey was distributed via email, Facebook, Whatsapp and GroupMe. The exact form of this survey can be found in the appendix. The survey consisted of 15 questions (13 multiple choice, 1 free-response and 1 scale question) in total, out of which 4 were demographic questions and 11 were research related questions.

> Results

The survey (see appendix) consisted of 36 participants of which 22 (63%) were males and 13 (37%) were females within the age group of 18 to 22 years. Of those surveyed, 63% were Asians, 20% White, 9% Hispanic and 9% others.

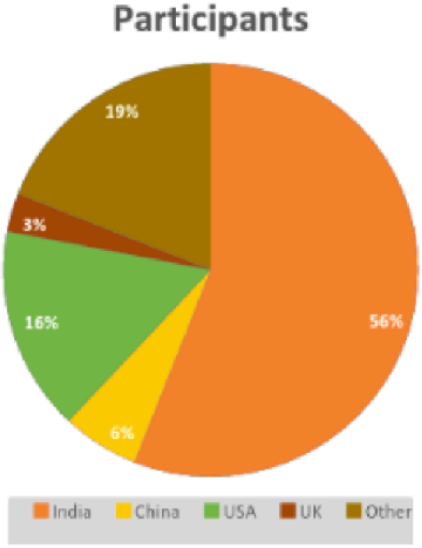


Figure 1: Percentage of participants by country.

Group A composed of 89% Indian and 11% Chinese students while Group B consisted of 83% American and 17% British students. *Figure 1* represents the percentage of participants from each country.

A notable observation was that 10% of Group A were not familiar with the concept of abortion compared to 0% of Group B. Moreover, 80% of the Americans and 100% of the students from the United Kingdom came across the term "abortion" at LEAST once a month while 82% of Group A came across the same term at MOST once a month. *Figure 2* compares responses given by students of each group with regard to abortion.

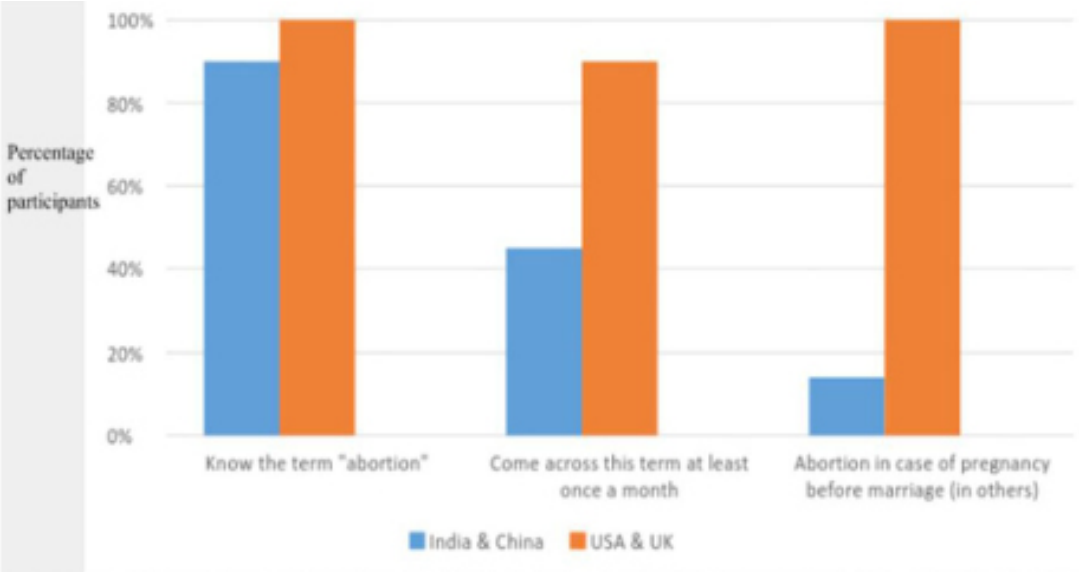


Figure 2: Graph representing result-abortion attitude in high power distance countries (Group A: India & China) and low power distance countries (Group B: United States of America & United Kingdom).

To the question, 'how many people they knew had considered abortion', most Indian and Chinese students stated 0 or 1 to 4, while 17% of Group B selected the option 5 to 10. 100% of Group B students claimed pregnancy before marriage as one of the reasons why people around them considered abortion and also 50% claimed it to be poor economic condition while 14% of Group A students stated pregnancy before marriage to be a reason. Group A students chose a wide range of options with 57% stating risk to health of mother or baby and 57% referred to poor economic condition.

29% of Group A and 33% of Group B students stated that they would consider abortion in case of pregnancy before marriage. 47%, 76%, 24% and 25% of Group A chose pregnancy as a result of rape, risk to health of mother or baby, poor economic condition and number of kids in family respectively relative to 67%, 100%, 33% and 17% of Group B students.

> Discussion

Abortion continues to be a controversial social issue amongst most families, societies and countries. As suggested by Bahr & Marcos (2003), there is a need for "comparative research" to identify factors influencing crossborder differences in abortion attitudes (p. 420). This study aimed to establish a relation between power distance practiced in a country and abortion attitudes, given an approximately similar education level. All participants were either high school or college students from India, China, the United Kingdom or the United States of America.

The results show that 10% of students from Group A were not familiar with the term "abortion". Further, students from Group B came across the term "abortion" much more frequently than those in Group A. This indicates less exposure and discussion of a sensitive topic like abortion in countries that practice high power distance.

17% of Group B knew of 5 to 10 people who had considered abortion compared to 0% of Group A which can be considered as an indicator to more liberal abortion attitudes and slightly higher abortion rates in countries practicing low power distance. The result that only 14% of Group A claimed pregnancy before marriage as a reason for abortion in others compared to 100% of Group B directs to conservative moral attitudes among students from countries practicing higher power distance. These observations are consistent with my hypothesis that power distance along with education influences an individual's opinion of abortion. But limitations exist as no participant was observed while taking the survey and the study was conducted on a small scale with disproportionate responses from each country making the data less reliable.

In their study, Wilson et al. (2011) and Scheepers et al. (2002) established that educational level and religious practices influence abortion attitudes. This impact of education is further supported in the research done by Bahr & Marcos (2003) and Wang (2014). My research study extends their idea and concludes that given an approximately equal level of education, power distance practiced in a country induces certain abortion attitudes. It is observed that low power distance culture leads to liberalized views of abortion while higher power distance induces more conservative views of abortion.

Even though high power distance practice shows a lower acceptance of abortion, these countries might have to expand their knowledge and discussing ability with respect to such topics. It is important to discover ways to increase discussion without inducing a rise in abortion rate in high power distance culture. One option would be to educate students of these countries intensively about other contraceptive methods while introducing abortion. In conclusion, preserving the high power distance culture might lead to decrease in abortion rate.

"Abortion" has been a much debated topic over the past decades and there exists several unexplored reasons behind varying abortion attitudes. Influence of parenting style, conversation orientation, conformity orientation within a family, childhood experiences and past relationship patterns on abortion attitudes are fields that require much more research.

> References

Bahr, S. J., & Marcos, A. C. (2003). Crosscultural attitudes toward abortion: Greek versus Americans. *Journal of Family Issues, 24*(3), 402424. doi: 10.1177/0192513X02250892

"Power Distance Index," (n.d.). Making sense of cross cultural communication. *Clearly Cultural.* [Web blog post]. Retrieved from http://www.clearlycultural.com/geert-hofstedeculturaldimensions/powerdistance-index/

Rutledge, B. (2011, September 18). Cultural Differences The Power Distance Relationship. [Web blog post]. Retrieved from http://thearticulateceo.typepad.com/my-blog/2011/09/culturaldifferencesthepower-distancerel ationship.html

Scheepers, P., Grotenhuis, M. T., & Van Der Slik, F. (2002). Education, religiosity and moral attitudes: Explaining crossnational effect differences. *Sociology of Religion*, *63*(2), 157176. doi: 10.2307/3712563

Scribner, S. (1984).Literacy in three metaphors, American Journal of Education *93*(1), 621. doi: 10.1086/443783

Wang, C. (2014). Induced abortion patterns and determinants among married women in China: 19792010. *Reproductive Health Matters, 22*(43), 159168. doi:10.1016/S0968-8080(14)437534

Wilson, K.S., Garcia, S.G., Olavarrieta, C.D., VillalobosHernández, A., Rodríguez, J.V., Smith, P.S., & Burks, C. (2011). Public opinion on abortion in Mexico City after the landmark reform. *Studies in Family Planning, 42*(3), 175182. doi: 10.1111/j.1728-4465.2011.00279.x

> Appendix

Survey Title: Literacy and Abortion

Q1. Age: _____

Q2. Sex o Male o
Female o Other

Q3. Ethnicity /Race o White
o Hispanic or Latino o African o
African American o Native American
o Asian o Other

Q4. Are you currently enrolled in college? o Yes o No

Q5. Education: What is the highest degree/ level of school you have completed? o Primary School Graduate o No schooling completed o Nursery school to 8th grade o Some high school, no diploma o High school graduate, diploma or the equivalent (for example: GED) o Some college credit, no degree o Trade/technical/vocational training o Associate degree o Bachelor's degree o Master's degree o Professional degree o Doctorate degree

Q6. Number of siblings excluding you
o 0 o 1 o 2
o Other (please specify): _____

Q7. How many kids do you or would you like to have?
o 0 o 1 o 2
o Other (please specify): _____

Q8. Are you familiar with the concept of abortion? o Yes o No
If No Is Selected, Then Skip To In which country did you spend most o...

Q9. How often do you come across this term outside of college? o Never o Less than Once a Month o Once a Month o 23 Times a Month o Once a Week o 23 Times a Week o Daily

Q10. How many people you know have considered having or have had an abortion?
o 0 o 1 to 4 o 5 to 10
o More than 10
If 0 Is Selected, Then Skip To Do you think a person should consider...

Q11. Which of the following reasons played an important role while they considered abortion as an option? o Pregnancy before marriage o Pregnant as a result of rape o Risk to health of mother/ baby o Poor economic condition o Sex determination of unborn baby o Number of kids in family o Effect on National Demographics (overpopulation, sex ratio etc.) o Others (please specify) _____

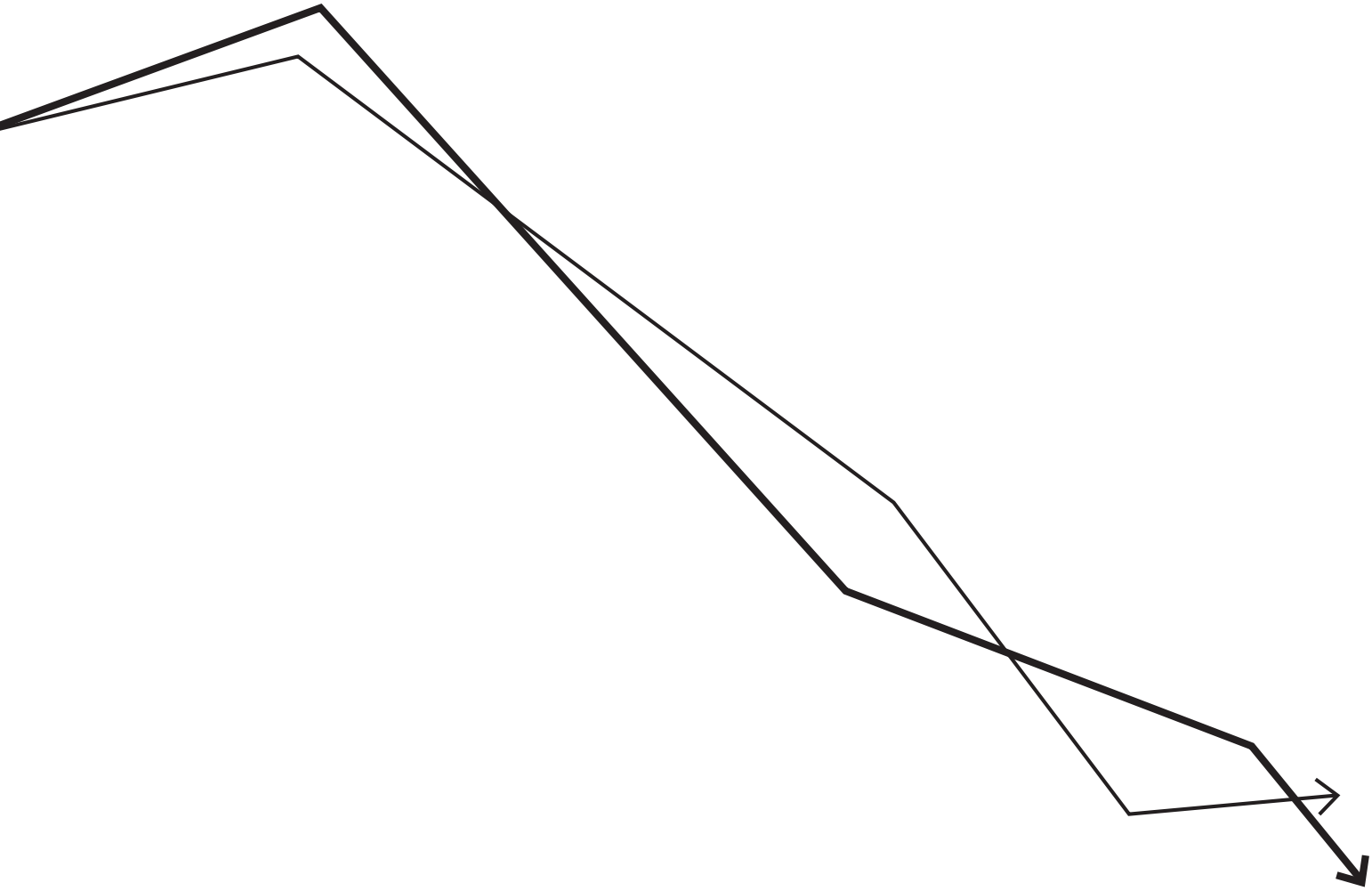
Q12. Do you think a person should consider other contraceptive methods (birth control pill, condoms, patch, IUD or emergency contraception i.e. Plan B)? o Yes o No

Q13. What are the chances that you would consider abortion? o 0 (Not at all likely) o 1 o 2 o 3 o 4 o 5 o 6 o 7 o 8 o 9 o 10 (Extremely likely)

Q14. You would consider abortion in case of (select all that apply) o Pregnancy before marriage o Pregnancy as a result of rape o Risk to health of mother/baby o Poor economic condition o Sex determined of unborn baby o Number of kids in family o Effects on National Demographics (overpopulation, sex ratio etc.) o Others (please specify): _____

Q15. In which country did you spend most of your life? (use the space below to elaborate) o United States _____ o China _____ o India _____ o Australia _____ o Other _____

REVIEW ARTICLES



THE EFFECTS OF ONCOGENES, EXPRESSION OF MICRORNAS, AND THEIR RESPECTIVE INHIBITIONS ON THE GROWTH, PROLIFERATION, AND METASTATIC PROPERTIES OF HEPATOCELLULAR CARCINOMA

by **Ashwin Ghadiyaram**

Ashwin Ghadiyaram is is a sophomore undergraduate student majoring in Biological Sciences at NC State University. He is a native of Tennessee and moved around quite a bit, having also lived in Oklahoma City, Oklahoma, for 6 years prior to moving to North Carolina when he was in the 4th grade. Outside of school, his hobbies are aviation spotting, watching basketball, playing basketball, eating good food with friends, and volunteering with the North Carolina Science Olympiad as well as WakeMed.

> Abstract

Hepatocellular carcinoma (HCC) is a cancer of the liver that is quite common and possesses a poor prognosis. Current treatments are ineffective at treating this specific form of cancer, but research over the last decade provides hope to finding more effective treatments in the future. The understanding of two primary variables related to the progression of hepatocellular carcinoma could lead to the discovery of a possible advanced treatment: oncogenetic activation/inhibition and increased/decreased microRNA (miRNA) expression. This literature review thoroughly explores both of these phenomena, their benefits, disadvantages, varieties, and effectiveness. The methods utilized by the groups involved the transfection of HCC cell lines expressing specific oncogenes and miRNAs into mice livers in order to observe the effect of the variables on the progression of HCC. Polymerase chain reactions (PCR) were used to transfect cells with desired genetic strands. Flow cytometry analysis (a technology that is used to analyze the physical and chemical characteristics of particles in a fluid as it passes through at least one laser) was used to identify the specific cell cycles cells are in. Decreased progression of HCC was found to be primarily associated with the inhibition of oncogenes and the increased expression of miRNAs. In contrast, the activation of oncogenes and the decreased expression of miRNAs had the opposite effect on HCC. These findings suggest that oncogenes and miRNAs have important roles in the outcome of HCC cell growth.

> Introduction

Hepatocellular carcinoma (HCC) is the most prevalent form of liver cancer. It is the fifth most commonly diagnosed cancer, and the third most common cause of death due to cancer (Zener et al. 2006). Treatment options, such as chemotherapy and radiation therapy, are not effective in treating HCC. This is probably why it has a high incidence of death. Furthermore, the cellular pathogenesis of HCC is currently not well known, which contributes to the difficulties associated with treating it (Liang et al. 2010). However, within the last decade, extensive research has been conducted on the genetic contributions to the proliferation of HCC, and how it can be inhibited. Research shows that oncogenes and microRNAs (miRNAs) play key roles, both negatively and positively respectively, in the progression and prognosis of HCC.

Oncogenes are genetic proteins that possess the ability to transform normal, healthy cells into cancerous ones as a result of genetic mutation. Once this progressive transition, also known as tumorigenesis, takes place, oncogenes often become expressed at high levels, contrary to normal expressive behavior. What makes tumorigenesis so dangerous to normal physiology is that apoptosis (programmed cell death of normal cells) is rendered inactive, leading to the uncontrolled growth and proliferation of cancerous cells (Zender et al. 2006). This eventually leads to death following metastasis (spreading of cancer cells by means of bloodstream from original tumor), if not treated. In addition, there are numerous genetic alterations that occur as a result of the evolution of cancerous cells, leading to their eventual instability. It is this instability that makes the advanced-stage cancerous cells highly adaptable to new environments, resulting in them being harder to treat (Zender et al. 2006). Zender et al. (2006), Ma et al. (2008), and Liang et al. (2010) specifically examined the effects of oncogenes on the tumorigenesis of HCC and how inhibition of particular oncogenes could alter the progression of HCC.

MiRNAs are small RNA (ribonucleic acid) molecules that are noncoding and regulate gene expression negatively by means of chemical interactions with corresponding messenger RNA molecules (mRNA). After these interactions take place, miRNAs form a conglomerate with the target mRNAs and reduce their protein levels, by either degenerating mRNAs or by hindering the translation of the target gene. Research indicates that miRNAs regulate approximately 30% of the genes in the human body, and may have important roles in human carcinogenesis (the initiation of cancer formation) (Liang et al. 2010). Kota et al. (2009), Liang et al. (2010), and Coulouarn et al. (2009) examined the effects of miRNAs on the progression of HCC.

Questions examined in this paper include: What specific effects do oncogenes and miRNA have on HCC tumorigenesis? What would their inhibition lead to in regards to the progression of HCC? Are there experimental trials that suggest any potential relationships between genetics and HCC?

> Effects of Oncogenes and Oncogene Inhibition on the Progression of HCC

In a recent study on the identification and analysis of oncogenes impacting HCC, Zender et al. (2006) utilized gene-expression analyses to evaluate the effects of the oncogenes, CIAP1 and Yap, on the onset of HCC. The method of studying CIAP1 involved first producing liver progenitor cells expressing CIAP1 by means of retroviral-mediated gene transfer (a form of transgenesis that utilizes retroviruses as vectors to transmit genetic material into a host cell). Then, in order to effectively determine whether CIAP1 could essentially function as an oncogene in vivo, the progenitor cells expressing CIAP1 were subcutaneously (under the skin) injected into nude mice. The study of Yap was compared to CIAP1 by injecting nude mice with Yap-expressing hepatoblasts in order to determine whether Yap could function as an oncogene in vivo. Precise measurements of tumor growths were taken in the days following the injections.

One major finding was that CIAP1 significantly accelerated the growth of hepatoblasts expressing myc (a known oncogene that alters cellular signal pathways, leading to the formation of liver tumors). This reduced the average onset time of liver tumors by approximately half, taking only 24 ± 2.3 days versus 45 ± 12.2 days ($p<0.05$) associated with myc oncogene expression without the effect of CIAP1. In addition, it was observed that CIAP1 significantly enhanced tumor burden (total tumor mass) with hepatoblasts expressing it compared to hepatoblasts not expressing it ($p<0.005$). Likewise, Yap exhibited a behavior nearly identical to CIAP1 in that it also accelerated liver tumor growth and increased tumor burden ($p<0.005$). As a result, Zender et al. (2006) concluded that CIAP1 and Yap possess oncogenic characteristics and accelerate tumor growth rates for HCC. However, in addition to both CIAP1 and Yap, there is another notable oncogene that has a profound impact on the carcinogenesis of HCC.

Ma et al. (2007) isolated and studied a novel oncogene, Amplified in Liver Cancer 1 (ALC 1), which they discovered to be amplified and overexpressed in the 1q21 DNA (deoxyribonucleic acid) band during the onset of HCC. In addition, they also studied the effects of the direct inhibition of ALC 1 on the progression of HCC by means of siRNAs (synthetic RNA duplexes designed to specifically target particular mRNAs for degradation). The method for studying the effect of ALC 1 on HCC involved procuring HCC cell lines from patients who underwent hepatectomy (excision of a cancerous tumor of the liver) due to HCC, performing chromosome microdissection to obtain five copies of the 1q21 band, amplifying the 1q21 band copies by means of polymerase chain reactions (PCR), and then isolating the ALC 1 gene within bacterial artificial chromosomes, which are DNA constructs used for transformations and cloning involving bacteria. The acquired ALC 1 was then cloned and used to form immortalized liver cell lines transfected with ALC 1. These cell lines were then utilized for 12 nude mice specimens, in which ALC 1 expressing cells were injected into the right dorsal flank of the mouse liver for six mice. Blank cells (the control), without ALC 1 expression, were injected into the left dorsal flank of the mouse liver for six other mice (control group). The effect of ALC 1 inhibition by siRNAs involved obtaining the H2-M HCC cell lines and transfecting them with double-stranded siRNA strands.

Following the injections of cell lines transfected with ALC 1, it was found that four-sixths of the mice that were injected with ALC 1 cell lines developed tumors in their livers while none of the mice that were injected with blank cell lines developed any observed tumors. Furthermore, the tumor cell sizes had an average volume of 464 mm3, which was significantly larger compared to the non-cancerous, blank cell line average volume of 88 mm3 ($p<0.001$). Furthermore, following the transfection of the H2-M cell line by siRNAs, the direct outcome was that two of the siRNAs were able to suppress ALC 1 , as evidenced by the cell lines exhibiting significantly highly reduced colony forming ability ($p<0.05$). The results of the mice liver trials support the tumorigenic potential of the ALC 1 gene on the pathogenesis of tumor cells within the liver. The results of the siRNA transfection trials clearly suggest that inhibition of the oncogene ALC 1 has a positive impact on the onset of HCC by effectively stunting its growth and proliferation. Overall, Ma et al. (2008) concluded that ALC 1 is an oncogene that supports tumorigenesis of HCC. In contrast, when inhibited, it results in the suppression of HCC proliferation. Although the role that oncogenes play in the development of HCC is well understood, miRNAs also have a profound effect on the progression of HCC, as well.

> Effects of miRNAs and miRNA Inhibition on the Progression of HCC

There is growing evidence indicating that miRNAs have an important role in the development of liver tumors. Kota et al. (2009) performed a thorough investigation regarding the delivery of therapeutic microRNA to hinder tumorigenesis in murine (rodent) liver cancer. They analyzed the efficiency of replacing miR-26a, a miRNA that is usually highly expressed in healthy cells, but experiences severely decreased expression in cancerous cells during the onset of HCC. MSCV (a murine stem cell virus) was injected into HepG2 cells (HCC cells) in order to enforce expression of miR-26a in HepG2 cells at a level similar to that of healthy cells. The control group utilized several miRNAs that support tumorigenesis. Afterwards, the HepG2 cells underwent flow cytometric analysis (laser-based technology used to count cells in particular cell cycles).

The flow cytometry data demonstrated that 69% of cells infected with MSCV - miR-26a were in the G1 cell phase ($p<0.05$) (phase in which cellular contents are duplicated), which was more than that of the control group cells. This implies that miR-26a expression induced a G1 arrest, effectively halting the growth of HepG2 cells. As a result, the proliferation of the HepG2 cells was also terminated, effectively stopping the progression of HCC.Kota et al. (2009) suggest that the delivery of certain miRNAs that are highly expressed by healthy cells into HCC cells can bring the cancerous progression to a halt. However, miRNAs can also be utilized to suppress HCC by means of directly targeting specific oncogenes.

In a study on the suppression of an oncogene by a specific miRNA, Liang et al. (2010) studied miRNA-125b and its effect on the oncogene LIN28B. Their methods consisted of extracting RNA and detecting mature miRNA-125b by means of reverse-transcribing the extracted RNA. Huh-7 cells (HCC cells) were then transfected into with miRNA-125b against LIN28B and then subcutaneously (under the skin) injected into nude mice between the ages of six and eight weeks. The control group consisted of mice injected with vector control cells without miRNA-125b. Furthermore, cell cycle distribution analysis was also conducted to assess the amount of

cells in each part of the cell cycle. Approximately four weeks later, the mice were manually killed and their tumors removed and weighed.

Mice liver tumor weight associated with miRNA-125b was significantly lower compared to that of the control group tumors ($p=0.0017$). In addition, the results of the cell cycle distribution analysis indicated increased cells at the G1 phase ($p=0.040$) compared to the cells of the vector control group ($p=0.004$). These outcomes indicated that miR-125b inhibited HCC tumor cell proliferation by directly inhibiting LIN28B. In addition, miR-125b blocked transition from the S phase to the G1 phase, thereby creating an arrest of the overall cell cycle at the G1 phase of HCC cells. Overall, the results of Liang et al. (2010) show that miR-125b suppresses HCC cell proliferation and metastasis by directly inhibiting the oncogene LIN28B, and that miRNA expression inhibits progression of HCC. In contrast, the absence of miRNAs could have an impact on the progression of HCC.

Coulouarn et al. (2009) examined the effect of a loss of expression of the microRNA miR-122 on the prognosis of HCC. Their method consisted of first obtaining 64 HCC cell tissues and 28 healthy liver tissues from patients undergoing partial hepatectomy for the treatment of HCC. The obtained HCC cell tissues represented various clinical features and outcomes. Consequently, the expression of miR-122 was assessed through reverse transcription (the reverse of normal transcription in which a sequence of nucleotides is copied from an RNA template during the synthesis of a molecule of DNA) by means of the mirVana quantitative reverse transcription-PCR miRNA Detection Kit and SYBR Green I (an asymmetrical cyanine dye that is utilized for staining nucleic acids for analysis).

It was found that miR-122 was repressed (not highly expressed) in the cells of HCC tumors that were characterized by poor prognosis (low chances of survival for the patient). It was also assessed that the survival time of HCC patients exhibiting low miR-122 expression was an average of 30.3 ± 8.0 months while HCC patients exhibiting high miR-122 expression survived for an average of 83.7 ± 10.3 months ($p<0.001$). Furthermore, the loss of miR-122 expression was related to a high occurrence of proliferation and low instance of apoptosis as portrayed by healthy cells. These results show that decreased expression of miR-122 contributes to the growth and metastasis of HCC cells, while abundant expression of miR-122 effectively counteracts the progression of HCC overall. Coulouarn et al. (2009) concluded that loss of expression of miR-122 is associated with a poor prognosis for patients afflicted by HCC, and a high expression of miR-122 functions to reduce the proliferation of HCC cells.

> Synthesis and Conclusion

All five studies that were reviewed support two main conclusions: the first being that oncogenes and the inhibition of miRNA expression both support the proliferation of HCC cells and their possible metastasis; the second being that inhibition of oncogenes and the presence of microRNA expression both inhibit the progression of HCC, effectively suppressing metastasis before it can occur. Zender et al. (2006), Ma et al. (2008), and Coulouarn et al. (2009) support the first point of oncogenes and inhibition of miRNA expression having a detrimental impact on the progression of HCC. Zender et al. (2006) primarily demonstrated that the genes CIAP1 and Yap both functioned to accelerate the rate of tumorigenesis of HCC cell lines, and were required for sustaining the rate, thus validating their characterization as oncogenes. In addition, the research associated with Ma et al. (2008) involved the identification of another notable gene, ALC 1, and assessing its oncogenetic characteristics. Trials were done on mice and the data suggested that ALC 1 lead to an increase in tumor volumes, directly related to the support of HCC cell proliferation. Furthermore, Coulouarn et al. (2009) investigated the effect of a loss of miRNA-122 expression on the outlook of HCC cell progression by assessing the expression of miRNA-122 in sample HCC tissues obtained from patients afflicted with HCC as well as healthy liver tissues. It was found that loss of miRNA-122 expression was associated with HCC cells that underwent aggressive growth and metastasis while healthy cells portrayed high expression of miRNA-122. These three research groups clearly portray that HCC cell proliferation and metastasis is indeed supported by the presence of oncogenes and the absence of miRNA expression.

On the other hand, Kota et al. (2009) and Liang et al. (2010) both bolster the second point of oncogene inhibition and miRNA expression having a positive impact on the advancement of HCC. Kota et al. (2009) had the primary goal of analyzing the effect of artificial miRNA delivery of miR-26a on the outlook of HCC. The team utilized stem cell viruses to administer miR-26a into a cell line of HCC and then proceeded to observe the effect of increased miR-26a expression in these cancerous cells versus the effect

of miRNA expression that supports tumorigenesis. Cell cytometry data went on to highlight that most of the cells expressing high levels of miR-26a expression were stuck in the G1 cell phase, leading the team to conclude that miR-26a expression effectively halts HCC proliferation by initiating an induced G1 cell phase arrest, stopping the progression of the cancer. Furthermore, Liang et al. (2010) studied the effect of miR-125b on the oncogene LIN28B as well as the overall progression of HCC. They studied the tumor growth in nude mice that were injected with miR-125b expressing cells that target LIN28B to inhibit it and with a vector control group of cells. The comparison of the tumor sizes led to the observation that miR-125b expressing cells experienced much less tumor growth than the latter, leading the team to conclude that HCC proliferation is effectively hindered by the expression of miR-125b. Also, cell cycle data showed that G1 cell phase arrest took place in the presence of increased miR-125b expression, as supported by the numerous number of cells in the G1 cell phase when the data was taken.

Although the research discussed does support the individual contributions of oncogenes and miRNAs to the progression of HCC, the studies collectively possessed two main limitations: the use of only mice livers as the primary specimens being transfected with cancerous cell lines, and the lack of variation of the mice specimens utilized. If animals other than mice could have been used, a sounder conclusion could be made. Furthermore, even among the mice liver trials, livers from various breeds of mice could have been utilized to provide more support to the evidence at hand. Overall, all of the research that was conducted could be applied to studying methods that could potentially inhibit the growth and proliferation of other types of cancer in addition to HCC, such as pancreatic cancer and lung cancer. In particular, a study on the effect of contact inhibition (deactivation by means of physical contact between one cell and another) on nonhuman primate HCC cell lines could be conducted to further contribute information on ways to counteract HCC growth and metastasis, in addition to the studies of oncogenes and miRNAs.

In conclusion, the five studies reviewed here demonstrate that both oncogene inhibition and miRNA expression suppress the growth, proliferation, and metastasis of Hepatocellular Carcinoma, with the exact opposite leading to its tumorigenesis and spreading.

> References

Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. 2009. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene. 28:3526-3536.

Kota, J; Chivukala, Raghu RR, O'Donnel KA., Wentzel EA, Montgomery CL, Hwang H, Chang, T, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. 2009. Therapeutic microRNA Delivery Suppresses Tumorigenesis in a Murine Liver Cancer Model. Cell. 137(6):1005-1017.

Liang L, Wong CM, Ying Q, Fan DN, Huang S, Ding J, Yao J, Yan M, Li J, Yao M, Ng IO, He X. 2010. MicroRNA-125b Suppressesed Human Liver Cancer Cell Proliferation and Metastasis by Directly Targeting Oncogene LIN28B. Hepatology. 52(5):1731-1740.

Ma N, Hu L, Fung JM, Xie D, Zheng B, Chen L, Tang D, Fu L, Wu Z, Chen M, Fang Y, Guan X. 2008. Isolation and characterization of a novel oncogene, amplified in liver cancer 1, within a commonly amplified region at 1q21 in hepatocellular carcinoma. Hepatology. 47(2):503-510.

Zender L, Sector M, Xue W, Flemming P. Cordon-Cardo C, Silke J, Fan S, Luk J, Wigler M, Hannon G, Mu D, Lucito R, Powers S, Lowe S. 2006. Identification and Validation of Oncogenes in Liver Cancer Using an Integrative Oncogenomic Approach. Cell. 125(7):1253-1267.

EFFECTS OF CANCER, CHEMOTHERAPY, AND DOMESTIC VIOLENCE ON FETAL DEVELOPMENT

by **Amaya K. Watters**
Mentor: **Lisa Paciulli, Ph.D.**

Amaya K. Watters is a junior in biological sciences at NC State University who intends to go to medical school after graduation. Amaya's research interests include fetal development, neonatal and maternal health, and oncology

> Abstract

Cancer, chemotherapy treatment, and domestic violence in utero can have negative effects on fetal development. Breast cancer complicates an estimated 1 in 3000 pregnancies (Hahn et. al 2006), ovarian cancer 1 in 8743 (Machado et. al 2006), and domestic violence affects about 6% (Yost et. al 2005). In a large study, 16,041 pregnant women were interviewed using four questions (had a partner hit, insulted, threatened, or screamed at her?) to determine the type of abuse, if any, they experienced during their pregnancies (Yost et. al 2005). The results were compared to investigate any differences among women who reported domestic violence and women who did not. In the case of fetal exposure to cancer or chemotherapy *in utero*, obstetric, pediatric, and oncologic data was obtained and examined for possible short-term effects (Calsteren et. al 2010). Long-term data for children exposed to maternal cancer and chemotherapy *in utero* was collected (Avilés and Neri 2001). Although women who reported domestic violence had adverse outcomes, those who declined to be interviewed were at higher risk for more adverse outcomes. On the other hand, children exposed to chemotherapy or cancer *in utero* generally had good short and long-term prognoses. In all studies, the most frequently observed effect was preterm birth and low birth weight. Adverse outcomes were due to preterm birth rather than the fetal environment itself. Cancer, chemotherapy treatment, and domestic violence have the same impact on fetal development: higher rates of preterm birth and neonatal intensive care unit (NICU) admission.

> Introduction

Maternal cancers and domestic violence are rare yet serious pregnancy complications. Breast cancer is diagnosed in about 1 in every 3000 pregnant women (Hahn et. al 2006), ovarian cancer in 1 in every 8743 (Machado et. al 2006), and domestic violence reported in 6% of pregnancies (Yost et. al 2005). There is a need to find the best way to treat cancers without causing damage to the developing fetus, and to determine how domestic violence affects the developing fetus. Fetal development is the beginning of all life, and therefore affects everyone. Understanding human development will help benefit quality of life as children progress through teenage and adult years. The fetal environment can accurately predict malformations, congenital abnormalities, or any medical complications that may be present at birth, and can foreshadow medical and psychological conditions at different life stages. It is important to pay attention to the fetal environment in order to prepare doctors and parents for possible birth conditions. Everything a pregnant woman does affects fetal development; the question is how and whether it is a positive or negative effect. This review article looks at the effects of cancer, chemotherapy, and domestic violence on fetal development.

The effects of domestic violence on fetal development were evaluated by Yost et al. (2005) in a large study with 16, 041 mothers. They asked four questions: had a partner or family member physically hurt her, insulted or talked down to her, threatened her with harm, or screamed or cursed at her during this pregnancy (HITS)? The neonatal outcomes were then compared between women who experienced abuse, women who denied abuse, and those who declined to interview. Domestic violence is a problem in pregnant women, but it has not been researched extensively and requires more future research.

In the case of maternal cancers, the effects of cancer and chemotherapy treatment on fetal development were studied. A higher incidence of maternal cancer is expected over the next few decades as more women delay childbearing until their 30s and 40s and pregnancy and cancer have more potential to coincide, so it is important that doctors are more prepared for this increase. In a recent study (Machado et al. 2007), the cases of 15 mothers who had ovarian cancer were analyzed. The maternal and neonatal outcomes of each case were evaluated and reported. This study is important because ovarian cancer is located at the origin of fetal development, and there is a need to know how the mother's eggs are affected and fetal development is influenced. A more recent study (Calsteren et al. 2010) collected data over a period of ten years on 215 patients and contributed additional data on neonates (newborns) affected by maternal cancers. This data further supports the findings of Machado et al (2006). Another study (Hahn et al. 2006) investigates how the treatment of breast cancer affects fetal development and children in their early lives. Using a sample size of 57 pregnant women with localized, invasive breast cancer and no previous systematic therapy, FAC treatment is conducted throughout the pregnancy and neonatal and child development is looked at later. This research gives clues as to how best treat maternal breast cancer with little to no adverse effects on the fetus, however, it lacks long-term results. Avilés and Neri (2001) do report the long-term effects of 84 children who were subject to chemotherapy in utero. The children were followed up from 6-29 years after birth to review their physical, psychological, and academic progress. This review paper looks at each of these topics in depth and discusses the impact they may have on fetal development.

> Effects of Domestic Violence on Fetal Development

In a 2005 study conducted by Yost et. al, the effects of domestic violence on fetal development during pregnancy were evaluated. Domestic violence was defined as actual or threatened physical, sexual, or psychological abuse between family members or intimate partners. This study looks at possible adverse pregnancy effects associated with domestic violence with an emphasis on birth weight.

Between December 6, 2000 and March 31, 2002, 16,041 women were approached to be interviewed. The interview was conducted by one of five bilingual, female research personnel not involved in the patient's care. They asked four questions: 1) had a partner or family member physically hurt her, 2) insulted or talked down to her, 3) threatened her with harm, or 4) screamed or cursed at her during this pregnancy (HITS) (Yost et. al 2005)? The interview was not conducted if the woman declined, had a stillborn, was about to deliver her baby, had a medical emergency, or could not be interviewed privately.

Within the study, 14,998 women (93%) denied any domestic violence, 949 (6%) reported domestic violence, and another 94 (0.6%) declined to be interviewed. Of the group that reported abuse,

women who answered yes to questions 1, 2, and/or 3 were put into the verbal abuse group. The remaining 137 (0.9%) women were put into the physical abuse group (Yost et al. 2005). Pregnancy outcomes were then compared among the four groups: no abuse, verbal abuse, physical abuse, and women who declined to interview.

The women who declined to interview were less likely to have prenatal care and experienced significantly higher rates of placental abruption, low birth weight infants, neonatal intensive care admission, and preterm delivery. Low birth weight was significantly increased by three times (12.8% versus 5.1%, declined-survey group vs. no-abuse group, respectively, P<.001) in women declining to be interviewed when compared with women denying domestic violence (Yost et al. 2005). Women who reported verbal abuse also experienced a significantly increased incidence of low birth weight infants (12.8% versus 5.1%, P < .001) when compared with those who reported no abuse (Yost et. al 2005). Women who reported physical abuse experienced a higher rate of neonatal death when compared to the no-abuse group (1.5% versus 0.2%, P = .004) (Yost et. al 2005).

Overall, women who declined to be interviewed were most at risk for adverse pregnancy outcomes. Women who do not respond to these questions may decline due to fear of retaliation by their abusers. It was found that low birth weight of infants was due to premature delivery as opposed to compromised fetal growth (Yost et. al 2005).

> Effects of Maternal Ovarian Cancer on Fetal Development

While the previous study looked at environmental effects on the fetus, this study evaluated the effects of maternal ovarian cancer on fetal development (Machado et. al 2006). Between 1987 and 2005, 131,149 babies were delivered at a hospital. Of these, 15 (.00011%) of the mothers were diagnosed and treated for ovarian cancer while pregnant. To determine the outcome of the neonates, birth weight, Apgar scores, gestation (time period between conception and birth) period, and survival rates were looked at (Machado et. al 2006). Apgar scores are performed at one, five, and ten minutes after birth to determine how healthy the baby is. There are five factors that are looked at, and each receives a score of zero, one, or two. These factors are appearance (specifically color), pulse, grimace (response to applied stimulus), activity, and respiration. Two is the best score and indicates a healthy baby. It is very rare for a baby to receive a score of ten at one minute; eight is a good score. A score lower than eight requires intubation.

The data collected on patients included pediatric, obstetric, and oncologic information, including obstetric history, symptoms, gestational age at diagnosis, gestational age and how gestation ended, and perinatal outcome (Machado et al. 2006). Patients were diagnosed by ultrasound (86.6%) or C-section at birth (13.3%). Four patients (26.6%) were diagnosed in the first trimester, seven (46.6%) in the second, two in the third (13.3%), and two (13.3%) during the puerperium (Machado et al. 2006). The puerperium is the six week period following parturition (birth).

Ten healthy term neonates (66.6%) were born with an Apgar score of ten at ten minutes old and adequate weight (Machado et al. 2006). Two (13.3%) neonates were born early: one at 33 and one at 35 weeks, both with good outcomes. The outcome of a very premature neonate with an Apgar score of 5 at birth weighing 1600 grams was unknown. There was one (6%) intrauterine death at 27 weeks and one (6%) miscarriage at week 14.

Ovarian cancer during pregnancy was rare with an incidence of 0.11/1000 deliveries, or 1 in every 8743 deliveries (Machado et. al 2006). This is a higher occurrence than what has been found in other studies, which may be due to this study being conducted at a hospital that maternal cancer patients were referred to. Two (13.3%) of the patients were treated with chemotherapy during pregnancy; both neonates were healthy and had no malformations after 1 year and 5 years respectively (Machado et. al 2006).

> Short-Term Effects of FAC Chemotherapy *in utero*

In a similar study, the outcomes of children who were exposed to chemotherapy *in utero* was evaluated (Hahn et. al 2006). Breast cancer is one of the most common malignancies diagnosed during pregnancy, with an estimated 1 in 3000 to 3 in 10,000 diagnoses being to pregnant women (Hahn et. al 2006).

In the study, 57 pregnant women with confirmed localized, invasive breast cancer not previously treated with systemic therapy were referred to a maternal-fetal medicine specialist for accurate

assessment of gestational age using ultrasound. With the exception of one, patients were only treated in the second and third trimesters. They were treated with FAC chemotherapy: a 5-fluorouracil, doxorubicin, and cyclophosphamide routine. They were also given outpatient combination therapy through gestational Week 35 (Hahn et. al 2006). The gestational age they were exposed to FAC, number of cycles of FAC they were exposed to, and total dosages of FAC they were exposed to were recorded (Hahn et. al 2006).

There were no stillbirths, miscarriages, or perinatal deaths. In the study, 54 (94.7%) patients delivered at a gestational age of at least 34 weeks, and the remaining three delivered before 34 weeks. Only one child weighed less than 2000 grams at birth while 6 weighed less than 2500 grams at birth (Hahn et. al 2006). Once the children were aged 2 to 157 months, a survey was sent out to the parents and guardians of the children to track their developmental progress. The most common complication reported was difficulty breathing at birth, and 4 (10%) of the neonates required ventilation (Hahn et al. 2006). One child born at 38 weeks had a subarachnoid hemorrhage (bleeding in the space between the brain and the tissue covering the brain) on day 2 postpartum, neutropenia (low count of a type of white blood cell), and thrombocytopenia (platelet deficiency). One child had Down syndrome, and one had attention deficit disorder. With the exception of the child with Down syndrome, all children experienced normal development compared to siblings and the general population.

It was found that pregnant women with breast cancer can be treated with FAC chemotherapy in the second and/or third trimesters with little to no negative effects on the developing fetus (Hahn et. al 2006). In the study, 97% percent of the children experienced normal development and 43% had no health problems (Hahn et al. 2006). This study will need to be continued, and later follow-up will be required to know how the children exposed to chemotherapy *in utero* were affected in the long term.

An additional study focuses on the neonatal outcomes of 215 patients who were treated with chemotherapy during pregnancy (Calsteren et al. 2010). Data was collected internationally from 1998 to 2008 and analyzed to see how fetal development may have been altered. Patients diagnosed with invasive cancer during pregnancy were identified, and oncologic, obstetric, and pediatric information was obtained. The oncologic data included gestational age at diagnosis, obstetric complications, gestational age at delivery, and mode of delivery. Obstetric data included cancer type, date of diagnosis, and type and date of treatment. Pediatric data included birth weight, sex, congenital malformations, admission to the neonatal intensive care unit, and reason for admission (Calsteren et al. 2010).

The maternal age at diagnosis was 33.2±4.8 years and the gestational age was 21.0±10.8 weeks (Calsteren et al. 2010). Fifty-two (24.2%) of the mothers were diagnosed within the first trimester, 92 (42.8%) in the second trimester, and 71 (33.0%) in the third trimester. The most frequent tumor types were breast cancer (46%), hematologic malignancies (18%), and dermatologic malignancies (10%) (Calsteren et al. 2010). In five (2.3%) patients, a miscarriage occurred before cancer treatment was started. In 30 (14.0%) patients, the pregnancy was terminated at a gestational age of 10.9±6.8 weeks, and in 13 (6%) patients, pregnancy was terminated after a gestational age of 13 weeks.

Data on the gestational age at delivery was only available for 179 patients. The mean gestational age at delivery was 36.2±2.9 weeks. Fifteen (8.4%) patients delivered before 32 weeks, 82 between 32 and 37 weeks (45.8%), and 82 at term (45.8%) patients (Calsteren et. al 2010). In 26 of the children, their birth weights were below the tenth percentile for their gestational age (24.2%, P=.001). In 13 children, a physical abnormality was diagnosed at birth. Major malformations were observed in five (2.9%), and minor malformations in eight (4.6%) neonates. These are not increased incidences from the normal population. Eighty-eight (51.2%) were admitted to the NICU (Neonatal Intensive Care Unit), and for 75 (85.2%) children, this was due to premature birth (Calsteren et. al 2010).

The most notable effects of cancer on fetal development were high rates of preterm birth and NICU admission. 54.2% of children were born preterm (Calsteren et al. 2010). Patients receiving chemotherapy had an increased risk of preterm labor, which contributed to more medical complications than were observed in full-term pregnancies. Overall, the prognosis for pregnancies complicated by cancer was good.

> Long-Term Effects of Chemotherapy on Fetal Development

While the last two studies focused on short-term

effects of chemotherapy on neonates, the next study focuses on the long-term effects. Avilés and Neri (2001) reviewed the records of the National Medical Center and considered all women with hematological malignancies who were pregnant between 1970 and 1995. The long-term developmental progress of 84 children was tracked. The hematological malignancies included acute leukemia, malignant lymphoma, and advanced stages of Hodgkin's disease.

Follow-up data was collected 6-29 years after birth (median, 18.7 years) for the infants exposed to chemotherapy *in utero.* All 84 patients were evaluated by physical examination, complete blood count with differential and platelet count, serum chemistry, hepatic test, cardiac function, and chromosome studies on bone marrow cells (Avilés and Neri 2001). Complete neurological and psychological evaluations were performed by a physician. Academic performance information and learning deficiency data were obtained from schools (Avilés and Neri 2001).

Twenty-nine mothers had acute leukemia during pregnancy. Eleven mothers were treated with chemotherapy during the first trimester, 12 during the second, and 6 during the third (Avilés and Neri 2001). They had healthy neonates with similar weights and heights to the normal population; no congenital abnormalities were observed in the long-term. In addition, 28 of the children were living; one child died from an acute abdominal complication at nine years old. Before his death, he had experienced normal growth and development (Avilés and Neri 2001).

Additionally, 26 mothers had advanced stage Hodgkin's disease and were treated with chemotherapy during pregnancy. Ten received treatment during the first trimester, eight, during the second trimester, and eight during the third trimester. All neonates were normal in height and weight, and no congenital abnormalities were observed (Avilés and Neri 2001). Also, 29 mothers had malignant lymphoma during pregnancy; 17 were treated during the first trimester, eight during the second trimester, and four during the third trimester. All 29 children experienced normal birth weight and height with no congenital abnormalities; all are still living (Avilés and Neri 2001).

Overall, every child was healthy at birth and experienced normal growth and development (Avilés and Neri 2001). All cytogenetic material was normal and no cellular damage was observed. All neurological and psychological evaluations had normal results. No clear abnormalities in learning or behavior were observed. Their academic performances were also normal: two of the children were lawyers, two were engineers, and three worked in commercial fields after university. Forty-three children finished high school, 20 went on to university and 23 worked in technical areas. Twenty children finished elementary school, and 12 were attending different grades. It was found that the use of intensive chemotherapeutic agents in adequate dose intensity was not associated with excessive risk of fetal malformations (Avilés and Neri 2001).

> Discussion

Cancer, FAC chemotherapy, and domestic violence have the same impact on fetal development: higher rates of preterm birth and neonatal intensive care unit (NICU) admission. While some neonates were negatively affected, most had good developmental outcomes despite having unfavorable circumstances. Neonates are highly resilient to their environments and the majority develop into normal adults regardless of their fetal conditions (Avilés and Neri 2001). To assure the best possible neonatal conditions, it is best to delay birth until at least 37 weeks of gestation as medical issues arise when preterm birth occurs. When the neonates in these studies were admitted to the NICU, it was due to preterm birth which can be directly attributed to their fetal conditions.

Studies on cancer and domestic violence are difficult to conduct because not many patients meet the criteria, and of those who do, not all are willing to participate. Maternal cancers are rare, and pregnant women who are victims of domestic violence are the minority. Many victims of domestic violence are not willing to admit it due to embarrassment or fear of retaliation from their abusers, and therefore may be more difficult to find (Yost et al. 2005). Therefore, it is safe to assume that some of the women who denied any abuse may have actually been involved in an abusive relationship. As a result, further research is needed in these specific topics.

A specific study that could be investigated is how the placenta serves as a biological protector for the developing fetus. It is clear from the results that cancer very rarely metastasizes from the mother to the placenta or fetus, and that chemotherapy treatment drugs are not absorbed through the

placenta or umbilical cord in amounts significant enough to cause substantial fetal damage. How does the placenta protect the fetus so well? What mechanisms exist that prevent a higher frequency of transplacental metastasis? What mechanisms are in place that protect the fetus from damage by chemotherapy drugs, given that the umbilical cord shares substances between the mother and fetus? One would assume that some of these drugs would be absorbed by the developing fetus and it would result in a higher rate of stunted growth or intrauterine death by overdose, but this was not observed in any of the studies. The results supported the use of chemotherapy as a method of treatment during pregnancy. The placentas of mothers who were treated with chemotherapy during pregnancy could be examined by a pathologist and tested for toxins. A control group of placentas not exposed to cancer or chemotherapy could be compared to placentas that were, and the differences in content and appearance could be recorded. Color, texture, substance levels, placental cells, obstetric data and oncologic data could be collected to determine what provides such immunological effects.

> Conclusion

Cancer, chemotherapy treatment, and domestic violence can have negative effects on fetal development and neonatal health. They may cause preterm birth, which can result in a myriad of other health issues. FAC chemotherapy treatments are safe to use in most cases and when approved by a doctor (Hahn et al. 2006), but children exposed to chemotherapy *in utero* do have a higher incidence of preterm birth. Cancer rarely metastasizes to the placenta or fetus, and most neonates develop normally despite having unfavorable circumstances (Machado et al. 2006). The outcome of neonates subject to cancer, chemotherapy treatment, or domestic violence *in utero* is generally positive and most will go on to lead normal lives.

> References

Avilés A, Neri N. 2001. Hematological malignancies and pregnancy: a final report of 84 children who received chemotherapy *in utero*. Clinical lymphoma 2(3): 173-177.

Calsteren K, Heyns L, DeSmet F, Van Eycken L, Gziri MM, Van Gemert W, Halaska M, Vergote I, Ottevanger N, Amant F. 2010. Cancer During Pregnancy: An Analysis of 215 Patients Emphasizing the Obstetrical and the Neonatal Outcomes. Journal of Clinical Oncology 28(4): 683-689.

Hahn K, Johnson P, Gordon N, Kuerer H, Middleton L, Ramirez M, Yang W, Perkins G, Hortobagyi G, Theriault R. 2006. Treatment of Pregnant Breast Cancer Patients and Outcomes of Children Exposed to Chemotherapy *in Utero*. Cancer 107(6): 1219-1226.

Machado F, Vegas C, Leon J, Perez A, Sanchez R, Parrilla J, Abad L. 2006. Ovarian cancer during pregnancy: Analysis of 15 cases. Gynecologic Oncology 105(2):446-450.

Yost N, Bloom S, McIntire D, Leveno K. 2005. A Prospective Observational Study of Domestic Violence During Pregnancy. Obstetrics and Gynecology 106(1): 61-65.

A GENERAL REVIEW OF RESEARCH ON VARIATION IN HUMAN HEIGHT

by **Taylor K. Nguyen**
Mentor: **Lisa Paciulli, Ph.D.**

Taylor K. Nguyen is a junior studying anthropology and evolutionary biology at NC State University, and is an aspiring biological anthropologist. She first began her interests the natural sciences as a child who often visited the Field Museum of Chicago. In the future, Taylor hopes to one day work as a curator for a museum or zoological institute as well as continue to advocate for conservation of species, specimens, and artifacts.

> Abstract

Human stature is a trait that is varied throughout many populations around the world. The variation in human height is found through many different determinants that fall into two umbrella categories, genetic and environmental. In order to study which factors affect adult height, studies on both categories were compared and analyzed. This review paper is primarily about the environmental and genetic factors of height variance in people of European descent. Since individuals of European descent tend to be genetically similar, it is ideal to use them in these studies. The research articles consist of longitudinal, cohort, and crosssectional studies. It was found that overall, genetic factors were more predictive in determining mature adult height than environmental factors. Finding variables related to height are important to know because if we are able to pinpoint the exact determinates of this trait, we may be able to influence height of future generations. In addition, we may be able to alter the effects height has on certain diseases.

> Introduction

Human height is a polygenic inherited trait, and many factors determine its phenotype. The study of human variation is important because variation is what causes us to thrive and adapt as a species. By studying the variation of human height, we can determine what factors make us become the most biologically fit. We can also study diseases or mutations that are linked to height . When studying height variation, Genomewide association studies (GWAS) are typically used to analyze genetic variants in a sample that may be linked to height. Single nucleotide polymorphisms (SNPs) are also used to compare deoxyribonucleic acid (DNA) sequence variants among a sample that shows linkage with the height trait. As for environmental factors of this trait, height usually is measured physically by a medical practitioner, and subjects are given surveys to fill out related to their personal lives (Rona *et al*. 1978).

In this review, many questions about factors affecting height variation will be addressed. Some variables affecting height include the genes that attribute to height variation, how prevalent certain factors are in determining mature stature, and how people in different societies vary in height. Some points that are discussed include the major and minor determinants of height variation and how they are studied (Smith *et al*. 201X). Studies included in this review were chosen based on the factors that possibly dictate human height. One study examined the Ctype peptide natriuretic peptide (a molecule that regulates bone and cartilage growth) and which genes are associated with body height (Estrada *et al*. 2009). Another study focused on "missing" heritability, or variants that are insignificant and are usually overlooked by using height to measure phenotypic variance (Zhang et al. 2012). In a gene linkage study, Liu *et al*. (2003) suggest that there are regions on the X chromosome that carry genes that are connected to variation in human height. In addition to heritability and the genetic factors of human height, studies conducted on the environmental aspects of height variation are included in this review. A study conducted on twins that compared stature from different countries explores how environmental factors play a role in determining adult height (Silventoinen et al. 2003). Adolescent height is also analyzed by Rona et al. (1978), when they look at how socioeconomic status showed a link in stature throughout the United Kingdom. Through these studies, physical issues and societal impacts regarding stature in humans can be further examined.

> Studies

Ctype Natriuretic Peptide and Height Variation

Human body height is a quantitative trait, and both genetic and environmental factors affect it. Therefore, studying the origins of these factors, as they relate to height is important. In order to study heritability, or the genetic factors of height variation, genetic information was taken from a sample of a European population in order to find a link to height. Estrada et al. (2009) conducted a genomewide association study of body height that suggests variation in the Ctype natriuretic peptide (CNP) signaling pathway has a significant role in determining human height by genotyping. The sample consisted of Northwestern Europeans, as their height has mostly stabilized at a biologically resolved maximum. The sample size used for this study consisted of 10,074 individuals. Using genotypes found in the HapMap Project (Haplotype map of the human genome) and other genotyping platforms, 2543,888 single nucleotide polymorphisms (SNPs) were found. The relationship between height and SNPs were assessed using sexspecific, agespecific residuals that were analyzed under an additive genetic model. Combined analysis of the sample found SNP rs6717918 on chromosome 2q37.1 as being associated with body height at a genomewide significant level of P= 3.4 x 10 ^9 (Estrada et al. 2009). Many SNPs in this region were found to be correlated with human height and were only in relatively weak linkage disequilibrium (LD). In the 2q37.1 region, the most significant evidence for association with stature was for rs749052, which had P= 1.4e6. It was suggested that the NPPC gene (Gene for the Ctype natriuretic peptide protein) may act as a clearance receptor modulating the effect of CNP (Estrada et al. 2009). Provided the data, it was concluded that the recognition of SNPs near the NPPC and the NPR3 genes having a strong relationship with human height points to a prominent role of the CNP signaling pathway in human height variation.

Genes on the X Chromosome and the Link to Variation

Another study that shows significance of gene linkage to height variation analyzes genes specifically on the X chromosome . In Liu *et al*.'s (2003) replication study on genes in the X chromosome that suggest a link to human

height, 1,816 people of 79 different pedigrees of European descent were tested to see whether Xq2425 (region on the X chromosome) is linked to adult height. This study was initially conducted to scan the human genome for quantitative trait loci (QTLs) underlying height variation in 630 subjects of 53 different pedigrees. The subjects were found through the Osteoporosis Research Center of Creighton University and 630 of the subjects were used again from the previous study. Subjects with chronic disease involving vital organs were excluded from the study, and recruitment of the sample was based on bone mineral density. Twenty ml of blood was drawn from each subject and inserted into EDTA (ethylenediaminetetraacetic acid) containing tubes. The blood was then stored in a chilled environment for five days, DNA was extracted using a Puregene DNA isolation kit (kit used to purify and isolate DNA), and finally the DNA was genotyped. A genetic database management system was also used to control the phenotype and genotype data for linkage analysis. In linkage analysis, age and sex were used as covariates for height, because they typically affect height variation. The results were that the Xq2425 region consistently showed a link to height in all of the subjects. A Logarithm of Odds (LOD) score of 2.66 at the marker DXS8067, which showed a significant linkage to height ($p< .05$). Another region on chromosome 5 (5q31) had an LOD score of 0.96 (Liu et al. 2003). To further validate the significance for the linkage of Xq2425 and 5q31, they computed multipoint LOD scores in 26 of the pedigrees added to the present study. A two point LOD score of 1.00 was achieved for the region Xq2425 (p= 0.0197) and a score of .72 was found for 5q31 (p= 0.048) (Liu et al. 2003). Typically, a LOD score of 3 is widely accepted to be statistically significant for linkage in a whole genome scan, so no general agreement was reached. Although further research is needed for confirmation of these results, a 2.66 LOD score can be considered significant for this study (Liu et al. 2003). Because of all of the different pedigrees included in the study, Liu et al (2003)'s study was highly suitable for genome linkage. Since this was a replicated experiment, the data Liu et al. (2003) found was highly significant because the 26 additional pedigrees still showed a relationship between Xq2425 and height, as the previous study had found.

Less Significant Loci and Heritability

Although the studies that were already reviewed evaluated more common genes that affect height variation, the next study considers the lesser known genes that may be just as relevant in determining height. A recent study was conducted where missing heritability and less notable loci were found that had an association with variation in human height (Zhang et al. 2012). A sample of 1,304 individuals from Hvar Island outside of Croatia was examined by assessing the extent of height variance shown through different SNPs. An analytical approach that estimates allelic effects and dissects allelic heterogeneity from GWA summary data was used instead of demanding individuallevel information. Height summary data were used from The Genetic Investigation of Anthropometric Traits (GIANT). These studies were used to examine SNPs to define physical adjacency to one another with 565 males and 739 females (Zhang et al. 2012). Blood samples and anthropologic data were taken from these subjects and yielded a genotype dataset of 2.5 million SNPs. In order to evaluate accuracy of this procedure,
the results of the estimated effect sizes and allelic heterogeneity were compared with the reported effect sizes in the GIANT summary data, which had a close relationship. By using the HapMap project, allelic heterogeneity of significant loci associated with height was evaluated. The allelic heterogeneity revealed that half of the secondary SNPs occurred within 200 kb from the primary SNPs, there was not consistent LD between primary and secondary SNPs, and there was no correlation between the estimated effect sizes of the primary and secondary SNPs (Zhang et al. 2012). The extent of variance in age and genderadjusted height using genetic scores based on the estimated allelic effects of the clustered significant loci. (Estrada et al. 2009)About 30% of variance was explained by secondary and tertiary SNPs. It was concluded that although primary and secondary SNPs of a single locus tended to cluster together, there was no clear correlation of effect sizes between these SNPs (Zhang et al. 2012). The results confirmed that loci with lower significance levels, and accounting for many variants at a locus, greatly increased height variance.

Twins and Geographic Location

Despite the importance of genetic factors in height variation, environmental factors also play a role in the determination of human height. Silventoinen *et*

al.'s (2003) study on heritability of adult body height used pairs of twins from eight different European countries in order to analyze geographical effects on human height. Data from 30,111 twin pairs were analyzed by using a statistical modelling package. Because there are very small differences in the genetics of Caucasian population, this sample was ideal in testing the environmental factors of height (Silventionen *et al.* 2003). In seven countries, height measures were based on self-report from questionnaires, while data collected from the UK was based on measured height using a stadiometer. A model that assumed that phenotypic variation can be due to variation in additive genetic, dominance genetic, shared environmental, and specific environmental components was tested, but shared environmental and specific environmental factors were excluded in the results of Silventoinen *et al.*'s (2003) study.

Average height in men ranged from 177 cm to 184 cm, whereas average height in women ranged from 163 cm to 171 cm by country (Silventoinen et al. 2003). Correlations for monozygotic (MZ) male twins in the score for this model were high in all countries. On the other hand, dizygotic (DZ) male twins were slightly more than one half of the MZ correlations.
Among men and women, average body height was lowest in Italy and highest in the Netherlands. Similar studies have shown this same correlation, which shows that people in Southern Europe are generally shorter and people in Northern Europe are generally taller. The reasons for this pattern were not fully understood, but genetic factors are typically most important when interpreting geographical differences in height. Shared environmental factors exhibited a strong relationship to variation in body height in only one of the male populations, whereas in women it was more prevalent. The stronger effects of environmental factors of height in women were more important due to the differences in the shared environment in their lifestyle. In conclusion, phenotypic variation and the genetic architecture of human height are similar in full populations in spite of substantial variation.

Socioeconomic Status in Adolescents

In addition to height stature in twins, different aspects of height factors were explored in adolescents. In an early study on the social economical aspects of the factors that affect height, primary school children and their parents were examined to see if there was a correlation between height and social status. This study was conducted in England and Scotland by randomly sampling different areas and targeting various areas of lower economic status. Height was analyzed by a Standard Deviation Score (SDS) by using the univariate genetic model of the Mx statistical package which calculated the difference between height and mean height of a population of the same age and sex. The modeling package then divides the mean by the standard deviation for that particular population. The sample size consisted of 9,815 children aged 5 to 11 years, and their parents. Fathers of the children's social class, sibship size (familial group size of same paternity), employment status, birth weight, mother's age, and parental height were all the factors considered in this study. Every child in the sample was measured by either school nurses, or other health professionals. In addition, two weeks before each measurement, parents were given questionnaires to fill out. Height was measured in 99% of the children, whereas 92% of questionnaires were completed. Accuracy of the questionnaires was tested by comparing the responses to birth weight to data provided by that area's health authority. To test accuracy of parental height, 50 parent couples were physically measured without notice. The study reported that height of mothers tended to be more accurate than those of the fathers. The children of white-collar workers tended to be taller at most ages, and on average were 30 cm taller than girls of bluecollar workers (Rona et al. 1978). In similar social classes, English boys had a tendency to be taller than Scottish boys. The average difference in social classes for SDS was about 0.6 for England and 0.5 for Scotland. As for the number of siblings per family, height tended to be lower in larger families, especially in those whose fathers were manual workers. This could be due to food distribution, as the larger families may not be obtaining as much nutrients as smaller families are. In unemployed families versus employed families, it was found height was considerably lower in families whose head was unemployed. The age of each child's mother at the time of birth was factored in, but showed no strong correlation to height variance. Height of both mother and father had the most distinct association with attained height in England and Scotland. The results in both countries were similar and had a 30.8% and 31.1% of variation for England and Scotland, respectively ($p > 0.05$)

(Rona et al. 1978). In England, the strongest economical factor with height was social class whereas in Scotland, unemployment was most associated with height. The findings of this study show that people in lower classes were shorter and people in these classes whose families were unemployed are shortest.

> Conclusion

Overall, we can conclude that there are many factors that contribute to human height, and some are more relevant than others. In the studies described, genetic factors appear to be more significant in determining human body height than environmental factors. In Estrada et al.'s (2009) study on Ctype natriuretic peptides, the data show that specific SNPs near certain genes (NPPC and NPR3) have a significant association with the etiology, or cause, of human height variation. A replication study conducted on gene linkage in the X chromosomes confirms a significant linkage from the region Xq2425 to human body height (Liu et al. 2003). In Zhang et al.'s (2012) study on missing heritability, it was determined that while taking in multiple variants at a locus into consideration, loci with lower significance levels showed that height increased significantly. The results from the studies analyzing genes seem to imply that height has a strong link to genetic inheritance. The comparative study on twins in eight different European countries that analyzed the difference in height between affluent countries (Silventoinen et al. 2003), found that overall, heritability in height was lower in women, and geographic variation was higher among men. These results show that men tend to inherit height more than women, which also further demonstrates gene heritability on the X chromosomes. Of the environmental aspects considered, we only see a slight increase in height. Rona et al.'s (2003) study on socioeconomic status on adolescents and their parents found that socioeconomic status played a significantly small part in height variation. Despite these results, it was concluded that people in lower classes do tend to be shorter on average.

Due to the diversity in different societies around the world, it is hard to pinpoint the exact determinants of human height. Nutritional status and diseases are very prominent in controlling human linear growth. However, significant familial aggression of human height corresponds to heritability above 50% which dominates variation in body height (Liu et al. 2003). The data seem to indicate that height is based more on heritability than any other factor. Even though it is shown that genetics are very important in determining someone's height, it is possible to influence a child's stature by nutrition. Despite their paternal genes, children have the ability to become taller than their estimated mature height, given the proper nutrients. This can be affected especially during critical growth periods of the adolescent. Although these studies all show significant results, there were some limitations in the experiments. A common problem in the studies that were testing the genetic factors (*e.g.*, Estrada et al. (2009), Liu et al. (2003), Zhang et al. (2012)) was the lack of personal information known about the subjects that may have affected the results. In Silventoinnen et al.'s (2003) study, the limitation was the lack of taking the nutrition of the subjects into consideration of the results. Another limitation, specifically in Rona et al.'s (1978) study, was the low response rate for particular questions regarding income on the surveys given to parents. Despite these limitations, the subjects chosen were ideal for these studies. Since Europeans tend to be genetically similar, more accurate results could be achieved through each study. Although these studies portray variables that affect height well, it would be beneficial if there were additional studies that delve deeper into these factors. If possible, a longitudinal study involving intake of certain macro and micronutrients that affect adult stature of similar genetic backgrounds could be conducted to further research height variation. It could also take different groups of individuals from around the world in order to see some results of height variation from non-Caucasian communities. This particular experiment may be able to help scientists study deeper into diseases from nutrient deficiency. Although there are advantages and disadvantages to being both short and tall, thanks to variation, we as humans have been able to adapt to certain environments, and thrive as a species. Human body height offers us an opportunity to further research how possible diseases, their treatments, and societal behavior affect humans physically. Discovering the factors that go into determining variation in body height could also help us pinpoint issues related to this trait, and could even allow us to determine the exact height of future generations.

> References

Estrada K, Krawczak M, Schreiber S, Dujin K, Stolk L *et al* . 2009. A genomewide association study of northwestern Europeans involves the Ctype natriuretic peptide signaling pathway in the etiology of human height variation. 18(18): 35163524.

Liu Y, Xu F, Shen H, Deng H, Liu Y *et al.* 2003. Conformation linkage study in support of the X chromosome harbouring a QTL underlying human height variation. 40: 825-831.

Rona R, Swan A, and Altman D 1978. Social factors and height of primary schoolchildren in England and Scotland. 32: 147154.

Silventoinen K, Sammalisto S, Perola M, Boomsma D, Cornes B *et al.* 2003. Heritability of adult body height: a comparative study of twin cohorts in eight countries. 6: 399408

Zhang G, Karns R, Sun G, Indugula S, Cheng H *et al.* 2012. Finding missing heritability in less significant loci and allelic heterogeneity: Genetic Variation in Human Height. 7(12): 17.

A REVIEW OF THE CAUSES AND TREATMENTS OF DEMENTIA

by **Millie Hair**
Mentor: **Lisa Paciulli, Ph.D.**

Millie Hair is a biology major at NC State University interested in conservation and will be participating in an internship program at the Duke Lemur Center during Summer 2017. Millie wrote this review at the start of sophomore year after having just spent the summer as her grandmother's caretaker after her husband passed away. She has dementia, and was the inspiration behind this literature review.

> Abstract

Dementia is a broad category of diseases dealing with loss of cognition and memory. Dementia comes in many forms, and is an ever-growing problem. There is no cure for dementia, as it is an irreversible condition. Studies researched treatment options using *Salvia officinalis* to control choline levels in the brain by comparing the levels before and after patients used the medicinal plant. Another study explored treatments using therapy animals by observing changes in patients attitudes when exposed to various dog stimuli. Little is known about the causes of some forms of dementia, but information was gathered in studies to see what causes early-onset dementia, and how complicated dementia can affect patients' lives. Another possible cause leading to possible treatments was explored in a study that tracked changes in cerebral glucose metabolism over the course of one year. This review sought to learn how dementia is caused and what can be done to treat it. Some possible treatments were found, but further research is needed.

> Introduction

Dementia, contrary to popular belief, is not a disease. It is a term describing a wide range of symptoms, like memory loss, or simply a decline in mental intelligence that affects the ability to carry out daily functions. The main diseases that fall under the category of dementia are Alzheimer's disease, Vascular Dementia, Lewy Body Dementia, and Frontotemporal Dementia. These types of dementia are progressive, meaning they worsen with age and are irreversible. Alzheimer's disease affects approximately 20 million people worldwide, making it the most common form of dementia (Akhondzadel *et al*. 2003). Alzheimer's disease is characterized in part by short term memory loss, behavioral changes, and unprecedented suspicions of family or friends (Alzheimer's Association 2015). Vascular dementia is the second leading cause of dementia behind Alzheimer's. It can be caused by a stroke, because the decrease in blood flow to the brain during a stroke can affect the portion of the brain that deals with memory (Nyenhuis and Gorelick 1998).

It is a common misconception that dementia only affects the elderly; but as one article in this review points out, while most symptoms of dementia don't develop until later in life, some people are diagnosed with early-onset dementia. "Early" is defined as less than 65 years of age. Early-onset dementia is important because the effects on the lives of patients/ patients' families can be more extreme. This is because younger people have more people relying on them; they could be not of retirement age yet or could still need to support a family. This puts more of a strain on anyone in the affected person's life than if the affected person were elderly (McMurtray *et al*. 2006).

Little is known about ways to prevent or treat dementia, because not much is known about the causes of dementia either. Since, as far as scientists know right now, dementia is irreversible, there is no current cure– only treatments and therapies. One article in this review discusses the prevalence of agitation and depression with dementia. These conditions lower the quality of life of a dementia patient, and therefore their causes should be explored so treatments can be found. Another article about the effect of therapy dogs focuses on how to improve the quality of life of dementia patients. This is important because since dementia is irreversible, once someone is diagnosed they can really only focus on coping or slowing down the progression. Therapy techniques can help with this process.

There is some research on the possible causes and treatments of dementia. One longitudinal study on cerebral metabolic decline tracks the progression of Alzheimer's disease throughout the brain, hoping to evaluate possible long-term treatments for Alzheimer's in its early stages. Cerebral glucose is necessary for the brain to function; it is synthesized to either ATP or glycogen which serve as main energy sources. The brain regulates the levels of cerebral glucose with complex neurons. Another study explored the possible treatments of dementia by testing whether the use of certain medicinal plants could treat symptoms. *Salvia officinalis*, a type of sage, has cholinergic binding properties and therefore could potentially treat dementia symptoms. Choline is a principle component of a neurotransmitter called acetylcholine. Since acetylcholine is involved in memory and learning, it is hypothesized that something that affects the synthesis of acetylcholine would also affect symptoms of dementia.

The goal of this review is to explore the research done on the causes and treatments of dementia. It is difficult to treat a disease if its cause is unknown, which is why these two go hand in hand. Each article is unique in its specific topic and methods, yet all of them are studies done to investigate either the causes of dementia or ways to treat dementia.

> Causes of Early-Onset Dementia vs Late-Onset Dementia

Less research is available about early-onset compared to late-onset dementia, so McMurtray *et al.*'s (2006) four-year study on the comparison of early-onset dementia (EOD) to late-onset dementia (LOD) was conducted to support the call for more research to be done on and to measure the frequency of EOD. Often more severe problems occur when dementia is early-onset, because of the likelihood that the patient still holds a job or has a family to provide for (McMurtray *et al*. 2006). To conduct this study, 1,683 patients were selected from a Veteran's Affairs Memory Disorder Clinic to participate in research about the causes and frequency of EOD. The patients' dementias and etiologies were diagnosed by neurologists, then demographic and dementia variables were compared between EOD and LOD. Of the 1,683 patients originally selected, 948 met criteria for dementia and 278 were early-onset.

It was found that EOD diagnoses were more often

from traumatic brain injury, alcohol abuse, HIV, and frontotemporal dementia– all of these showed statistically significant differences between EOD and LOD ($p \leq 0.001$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.05$, respectively). The LOD group had a much higher frequency of Alzheimer's disease than EOD– 60% compared to 30%. Vascular dementia was the most frequent dementia that was diagnosed before age 65, however LOD data also had a high frequency of vascular dementia. Thirty percent of the early-onset patients had conditions/etiologies that were more preventable or treatable, supporting the original thought that more attention should be paid to EOD because it can often be stopped (McMurtray *et al*. 2006). The population of this study is skewed towards low socioeconomic status, inner-city, white male veterans, because the patients were pulled from a Veteran's Affairs clinic (McMurtray *et al*. 2006). It may be a good idea to conduct more research on the prevalence of EOD in a more general population.

Since many of the causes of EOD are known, it could often be prevented; this is why it is important to explore the causes of dementia. Vascular dementia is one example where it is known that improving vascular health lowers the risk of getting dementia (McMurtray *et al*. 2006). There are many other possible causes of dementia that are being studied, one such example being the decline of cerebral metabolic rates (Alexander *et al*. 2002). Researching the effects Alzheimer's has on brain function can help lead to finding long term treatments that could be effective during the earlier stages of the disease to possibly slow its progression.

> Possible Cause and Treatment of Alzheimer's

Alexander *et al*.'s (2002) study hypothesized that Alzheimer's patients would show a metabolic decline in the regions of the brain affected by Alzheimer's disease over the course of one year. Fourteen patients were selected (twelve men and two women), with an average age of 65 and an average Mini-Mental State Examination (MMSE) score of 20. MMSE is a questionnaire on a 30-point scale that examines cognitive impairment, 1 being most cognitively impaired. There was also a group of 34 healthy subjects with similar demographics as the Alzheimer's patients; twenty-seven men and seven women, and an average age of 64. The main difference was that the "healthy group" had an average score of 29 on the MMSE. Subjects went through physical, medical, and neurological examinations to make sure the Alzheimer's patients did not have any other conditions that could affect the results of the study (Alexander *et al*. 2002). Patients had their eyes and ears covered, and head supported for the PET (positron emission tomography) scan used to map the brain. The cerebral glucose metabolism was measured after patients received injections of FDG (Fluorodeoxyglucose), a radiopharmaceutical used in PET scans. Arterial blood levels were also gauged for radioactivity and glucose concentration (Alexander *et al*. 2002).

The hypothesis was supported by the results of the study. Glucose metabolism levels in the posterior cingulate cortex, and in the parietal, temporal, occipital, and frontal regions of the brain were found to be lower in the group of Alzheimer's disease patients than with the healthy group (Alexander *et al*. 2002). The results of the one year follow up also supported the hypothesis as the Alzheimer's patients experienced a significant ($p \leq 0.005$) decline in glucose metabolism over time. The greatest decline after one year was in the frontal association regions (Alexander *et al*. 2002). Levels of other macromolecules in the body are thought to affect memory or cognition as well. Choline, for example, is a molecule within acetylcholine, which is involved in memory functions and muscle control. The levels of choline in the body affecting dementia has been studied before, and the results have led to a possible treatment in a medicinal plant (Akhondzadel *et al*. 2003).

> Treating Dementia Using a Medicinal Plant

Akhondzadel *et al*.'s (2003) study sought to evaluate the effectiveness and safety of using *Salvia officinalis* as a treatment for Alzheimer's disease. *Salvia officinalis* is a plant known to have medicinal values due to its cholinergic binding properties (Akhondzadel *et al*. 2003). *Salvia officinalis* demonstrates acetylcholine receptor activity. Acetylcholine is a neurotransmitter responsible for much short term memory function. The first neurotransmitter defect known to occur with Alzheimer's disease is acetylcholine (Akhondzadel *et al*. 2003). Acetylcholinesterase inhibitors, or AChEIs, have shown the most success out of all approaches to treat dementia with drugs that change choline levels in the brain.

This was a 40-month, parallel group, placebo-controlled trial. One hundred and three patients were involved in the study, 39 of which were randomly selected to receive medication. The patients selected

to receive medicine were randomly split using a computer between placebo and *S. officinalis* in a 1:1 ratio. The medicated patients were given 60 drops per day of their assigned medication. All other dementia medications were discontinued. The main efficacy measures were the ADAS-cog and CDR-SB (Akhondzadel *et al*. 2003). ADAS-cog stands for Alzheimer's Disease Assessment Scale- cognition, and is an evaluation often given by a phycologist to determine cognitive impairment. CDR-SB stands for Clinical Dementia Rating- sum of boxes, and is another test used to evaluate cognitive impairment (Akhondzadel *et al*. 2003). Outcomes of the study were measured by the change in ADAS-cog and CDR-SB scores throughout the trial. During the four-month trial, patients were assessed every two weeks by neurologists. A two-way repeated measures analysis of variance was used as a statistical analysis, along with a one-way repeated measures analysis of variance with a two-tailed post hoc Tukey mean comparison test (Akhondzadel *et al*. 2003).

There was no significant difference in demographics between the placebo group and the *S. officinalis* group. Weeks one and two of the study had nonsignificant differences in the ADAS-cog scores of placebo and *S. officinalis*. From week three on, the difference was significant. The change from the start point to the endpoint (16 weeks) of the study was -6.60 ± 1.63 (mean ± standard deviation) for the *S. officinalis* patients, and 5.53 ± 1.12 for placebo patients (Akhondzadel *et al*. 2003). In the analysis of CDR-SB scores, it was found that the first six weeks of the study showed no statistical significance, but there was for the remainder of the trial. The changes from baseline to endpoint were -1.60 ± 1.35 for Salvia extract and 0.73 ± 0.41 for placebo (Akhondzadel *et al*. 2003).

Patients that received *Salvia officinalis* treatment experienced statistically significant benefits in cognition after the course of the study. There was a drop in ADAS-cog and CDR-SB scores overall for the group taking the *S. officinalis* extract, showing cognition improvement and thus supporting the hypothesis (Akhondzadel *et al*. 2003). The data shows that the *S. officinalis* group had six times less agitation in their patients, possibly suggesting that the extract can lower agitation in dementia patients, however more research needs to be done. Agitation is actually one of the most common complications of dementia, along with depression (Bartels *et al*. 2003).

> Prevalence of Depression and Agitation in Dementia

Symptoms of agitation in dementia include wandering, restlessness, inappropriate disrobing, and verbal outbursts (Bartels *et al*. 2003). These symptoms of agitation are the most common reason for immediate placement into a nursing home. Depression in dementia is characterized by hopelessness, lack of energy/interest, and difficulty concentrating. These symptoms for agitation and depression make living with dementia especially difficult for the caregiver as well as the patient. The potential overlap of depression and agitation in dementia is warranted by the common responses patients, with or without dementia, have to similar pharmacological agents. For example, there is a serotonergic deficit in depression and agitation, so antidepressants that boost serotonin have similar effects on depression and agitation (Bartels *et al*. 2003). One-third of patients with complicated dementia (dementia with one or more other mental issue) have dementia with agitation, depression, or both (Bartels *et al*. 2003).

The sample size in this study was large- 2,478 residents in 109 different long-term care facilities were included; 1,836 met criteria for dementia. The average age of the subjects was 80. Criteria for this study included being "at risk" of an ulcer, and documented dementia by the Comprehensive Severity Index (CSI). Agitation was distinguished by meeting one of the following criteria: 1) Agitation NOS; mostly fidgeting, 2) Moderate Agitation; can't sit still, on edge, 3) Severe Agitation; violent, physical restraints necessary (Bartels *et al*. 2003). The data was collected from the nursing homes, long-term care facilities, and hospitals attended by subjects. The majority of dementia patients (56%) had dementia complicated with either agitation, depression, or both. The group that received the most medical drugs (6.7 ± 4.0) and psychiatric drugs (1.5 ± 1.1) was the mixed agitation/depression group. This group also had the highest rate of medical comorbidity (Bartels *et al*. 2003). A chi-squared test was used to analyze categorical variables, and the Kruskal-Wallis nonparametric analysis was used for continuous variables.

Although this study did not find a direct cause of agitation and depression in dementia, it did show that many dementia patients experiencing these symptoms could find relief with antidepressants. Depression and agitation are not paid as much

attention to as cognitive symptoms of dementia are. Treating these symptoms can improve a dementia patient's quality of life, as well as the lives of their caretakers, families, or friends. There is other research available on ways to improve quality of life. Marx *et al.*'s (2008) study on the impact of dog stimuli on dementia patients explores multiple methods and therapies for improving the quality of life of people living with dementia.

> Treatment Using Dog Stimuli

The goal of Marx *et al.*'s (2008) study was to evaluate the effectiveness of animal-therapy on patients with dementia. Dementia patients living in nursing homes spend 22% of their waking hours alone (Marx *et al.* 2008). A dog is a good companion for a dementia patient because they provide social interaction despite the cognition issues. However, it is not always practical to take a live animal into a nursing home, so robotic and plush animals were tested too.

The dog stimuli chosen to be used in the study were three real dogs, a robotic dog, a plush dog, a puppy video, and a picture of a dog to color. The three real dogs were three different sizes: a large standard poodle, a medium sized miniature schnauzer (25 lbs.), and a small miniature schnauzer (11 lbs.) (Marx *et al.* 2008). Fifty-six residents from two different nursing homes in suburban Maryland made up the sample size of this study. There were 44 women, 12 men, and the average age was 87. Sixty-three percent were widowed. Subjects were not included in the trial if they were diagnosed with bipolar disorder or schizophrenia, had no movement in their hands, were not able to sit comfortably, or were younger than 60 (Marx *et al.* 2008). These subjects' activities of daily living (ADL) score was measured using the MDS, or Minimum Data Set. The MDS is a federal assessment of functional capabilities of those in the Medicare system; it is a range from one to five, one being independent and five being completely dependent. The subjects in this study had an average score of 3.4 (Marx *et al.* 2008). Cognitive function was measured by the MMSE, with an average group score of 9.1.

Observational Measures of Engagement were recorded through direct observation. A presenter asked if the patient would like to participate in the activity, and then left the room. If the patient responded that they did want to participate, the stimuli would then be introduced and an observer would record the patient's attitude towards the stimuli and the duration of the experiment. Attitude was measured on a five-point scale of negative, somewhat neutral, somewhat positive, positive, or very positive (Marx *et al.* 2008). Duration was measured in seconds; if the patient declined the stimuli their duration was said to be zero seconds. Each activity lasted at least three minutes, after which the observer would stop the activity only when the patient stopped engaging. All engagements were stopped after 15 minutes.

The data was examined using the repeated measures of analysis, and Bonferroni test for subsequent analyses (Marx *et al.* 2008). The analysis of variance of engagement, duration, and attitude of the three sizes of dogs had statistically significant results. The stimulus that was most often refused was the small real dog, and the stimulus that was least often refused was the large real dog. Another analysis of variance was used to evaluate the difference between dog stimuli and non-dog stimuli, however no statistical significance was found. The dog stimuli compared to the control group of no stimuli at all resulted in 38 comments made by residents during activities with the dogs compared to only two made when there was no stimuli. Some limitations of this study include the handlers of the dogs; they could have influenced the patients' decision on which dog to engage with. There could have also been more than one breed of each sized dog, because the breed of dog could have also influenced which dog they engaged with (Marx *et al.* 2008).

> Conclusion

Dementia is an ever growing problem in the elderly population. There are many various causes of dementia, and some that are still unidentified. The same goes for treatments; while there is no way to reverse dementia, there are many treatments available to slow its progression or lessen its symptoms.

In the study conducted by McMurtray *et al.* (2006), early-onset dementia (EOD) was compared to late-onset dementia (LOD). McMurtray *et al.* (2006) examined the causes of EOD, and sought to bring attention to the fact that many of the causes can be prevented. EOD is often caused by head trauma, vascular problems, stroke, alcohol abuse, etc. One-third of the sample size for this study had EOD. EOD is often ignored in comparison to other dementias, in the same way that the complications of agitation and depression in dementia is sometimes discounted. Agitation and depression are common in dementia patients. Bartels *et al's.* (2003) study of agitation and

depression in dementia found that 20% of dementia patients also have depression. Over 1,000 dementia patients in nursing homes took cognition tests to determine the levels of agitation and depression. The purpose of this study was to bring attention to these important symptoms that aren't payed as much attention to as cognitive symptoms.

Marx *et al.* (2009) also focused on more behavioral characteristics associated with dementia rather than cognitive function. The study measured the effectiveness of animal therapy (particularly dog stimuli) on the attitudes of dementia patients. Dogs make a great companion for people with memory loss because they don't know when they've been told a story multiple times and will react with the same eagerness they would to any other verbal stimulation. The study found that the dementia patients responded well to the dog stimulus, but there was no significant evidence that the response was due to the dogs and the results wouldn't be the same for any other stimuli.

Alexander *et al.*'s (2002) study examined the effects of cerebral glucose metabolism on Alzheimer's disease. It used a PET scan to learn the levels of glucose in regions of the brain that are affected by Alzheimer's disease. This was a longitudinal study taking place over the course of one full year. After the year had passed, the original tests were taken again and results were compared. The results supported the hypothesis; Alzheimer's patients did have lower levels of cerebral glucose in the areas of the brain that deal with memory and learning after one year. Another study that looked at levels of chemicals in the brain is Akhondzadel *et al.* (2003). Acetylcholine is a neurotransmitter that deals with memory and cognition, and is therefore is tightly linked with dementia. Akhondzadel *et al.*'s (2003) study connected this concept to *Salvia officinalis*, a sage plant that is known to have cholinergic binding properties and some medicinal value. This study medicated one group with placebo and one with *Salvia officinalis* extract and compared the cognitive impairment of the two before and after the medication. The results showed that the patients who took the *Salvia officinalis* extract experienced an increase in cognition and lower agitation; the results were significant.

Dementia affects millions worldwide. There is a great deal of research being done on the causes and treatments of the diseases under dementia, however, more research is needed in order to make a substantial impact on the mortality rate and on the number of people affected.

>References

Akhondzadel S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. 2003. *Salvia officinalis* extract in the treatment of patients with mild to moderate alzheimer's disease: A double blind randomized and placebo-controlled trail. Journal of Clinical Pharmacy and Therapeautics 28:53-9.

Alexander G, Chen K, Pietrini P, Rapoport S, Reiman E. 2002. Longitudinal PET evaluation of cerebral metabolic decline in dementia: A potential outcome measure in Alzheimer's disease treatment studies. The American Journal of Psychiatry 159(5):738-45.

Bartels SJ, Horn SD, Smout RJ, Dums AR, et al. 2003. Agitation and depression in frail nursing home elderly patients with dementia: Treatment characteristics and service use. The American Journal of Geriatric Psychiatry 11(2):231-8.

Marx M, Cohen-Mansfield J, Regier N, Dakheel-Ali M, Srihari A, and Thein K. 2008. The impact of different dog-related stimuli on engagement of persons with dementia. American Journal of Alzheimer's Disease & Other Dementias.

McMurtray A, Clark D, Christine D, Mendez M. 2006. Early-onset dementia: Frequency and causes compared to late-onset dementia. Dementia and Geriatric Cognitive Disorders 21(2):59-64.

Nyenhuis, D. L. and Gorelick, P. B. (1998), Vascular Dementia: A Contemporary Review of Epidemiology, Diagnosis, Prevention, and Treatment. Journal of the American Geriatrics Society, 46: 1437–1448. doi: 10.1111/j.1532-5415.1998.tb06015.x

Types of Dementia [Internet]. 225 N. Michigan Ave., Fl. 17, Chicago, IL 60601: Alzheimer's Association; c2015 [cited 2015 10/2]. Available from: http://www.alz.org/dementia/types-of-dementia.asp.

> Acknowledgments

I would like to thank Dr. Paciulli for her guidance and assistance in the writing of this literature review. Her supervision made it all possible, and her passion for teaching and science was daily inspiration and motivation.

A LITERATURE REVIEW: EFFECTS OF ALTITUDE ON SEA-LEVEL PERFORMANCE AND THE HEART

by **Megha Ganatra**
Mentor: **Lisa Paciulli, Ph.D.**

Megha Ganatra graduated NC State University in Spring 2017 with a degree in Human Biology and a double minor in Anthropology and Psychology. She is currently conducting a research study at the Duke Lemur Center with ruffed lemurs on neonatal outcomes. Her research interests include exercise physiology and effects of cortisol.

>Abstract

Many athletes tend to invest a lot of resources into training at high altitudes. It is known that high altitudes induce erythropoiesis, the process of making red blood cells, which results in a higher oxygen carrying capacity. This has been shown to improve sea-level performance. Levine and Stray-Gundersen (1997) examined the effect of moderate-altitude acclimatization with low-altitude training on performance by exposing athletes to different altitudes. They found that living high and training low improves running performance at sea-level. Stray-Gundersen et. al (2001) further studied the effects of high altitude acclimatization and low altitude (HiLo) training using a modified training schedule. The HiLo model for training resulted in an improvement of sea-level performance in athletes. However, there was a large amount of inter-individual variation in erythropoiesis in response to altitude. To study this, Friedmann et. al (2005) examined whether increased hemoglobin mass could be predicted by erythropoietin responses to altitude in junior swimmers. Erythropoietin levels and hemoglobin mass were measured before and after altitude exposure. In addition, altitude induces physical and physiological changes in the heart. Carzorla et. al (2006) looked at how high-altitude exercise training affects contractile function by studying rats placed in different environments. They found that sea-level training improved the transmural gradient of stretch-dependent Ca2+ sensitization. Reboul et. al (2004) studied cardiac remodeling consecutive to altitude in rats with different training conditions, and found that altitude training resulted in left ventricular remodeling. In summary, high altitudes have a variety of effects including improvement in sea-level performance as well as physiological and physical changes in the heart.

> Introduction

In their constant search to improve performance, many athletes tend to train at high altitudes because hypoxia (deficiency in the amount of oxygen reaching tissues) exposure promotes erythropoiesis, the process of making red blood cells. This process, in turn, causes an increase in oxygen carrying capacity and thus an improvement in sea-level performance. Many earlier studies showed that endurance athletes may achieve some benefit from altitude training for sea-level performance. Levine and Stray-Gundersen (1997) reasoned that if athletes could live at moderate altitudes, but train at low altitudes (1250 m), then they could possibly acquire physiological advantages of altitude acclimatization for maximizing oxygen transport. In this study, Levine and Stray-Gundersen (1997) showed that acclimatization combined with low altitude training is superior to an equivalent training method at sea-level. To continue their early research, Stray-Gundersen et. al (2001) examined the effects of high altitude acclimatization and low altitude training on sea-level performance. Stray-Gundersen et. al (2001) showed that despite observing a significant improvement in sea-level performance, there were still some participants that were considered outliers. Friedmann et. al (2005) suggested that this was because there was high inter-individual variability in improvement in sea-level performance after training at moderate altitudes. Other studies have also found that there is a great inter-individual variability has been found in the erythropoietic (EPO) response to moderate altitudes. Friedmann et. al (2005) examine whether the EPO response to acute exposure to normobaric hypoxia has helped identify athletes who would respond to altitude training with an increase in EPO and hemoglobin concentration. Not only does altitude effect sea-level performance by promoting processes like erythropoiesis, but it also affects the heart physically. For example, Reboul et. al (2004) looked at how an altitude training camp affected ventricular morphology and function in rats. Aerobic exercise is known to induce morphological remodeling and functional adaptations that lead to an increased maximal stroke volume and cardiac output. In addition, Cazorla et. al (2006) studied the effects of high-altitude training on contractile function of rat cardiomyocytes (cardiac muscle cells). They examined the effects of training in different altitudes on the Frank-Starling mechanism, which states that the more the cardiac muscle is stretched, the stronger it will contract. This literature review will examine the effects of altitude on sea-level performance in athletes as well as the physical and physiological adaptations to the heart.

> Data Interpretation

Effect of moderate altitude acclimatization with low-altitude training

In an early study on the effects of altitude acclimatization during training, Levine and Stray-Gundersen (1997) observed the effects on training of athletes who lived in altitudes above 2,500 m, but trained at altitudes below 1,500 m. This study involved forty-one long distance runners that performed at the collegiate level. Thirty-nine runners, twenty-seven of which were men, were able to complete all the testing and training sessions (Levine and Stray-Gundersen 1997). All athletes were required to be competitive at a distance between 1500 m and a marathon, to have a personal best 5,000 m time, and to not have been to an altitude above 1500 m for a period exceeding one week in the 10 months prior to the study (Levine and Stray-Gundersen 1997).

This study design includes four major phases: sea-level lead-in phase, sea-level training phase, altitude training phase, and sea-level testing phase (Levine and Stray-Gundersen 1997). The sea-level testing period took place at 150 m (Dallas, Texas), and lasted approximately two weeks. It included supervised training at sea-level, which allowed all participants to be at an equivalent level of training readiness (Levine and Stray-Gundersen 1997). The sea-level training period lasted four weeks and involved supervised training at sea-level, with increased intensity of training each week, and repeated laboratory testing (5000 m time trial). In the third phase, the athletes were matched for gender, 5000 m time trial performance, and training history into groups of three and then randomly assigned to "high-low," "high- high," or "low-low" groups (Levine and Stray-Gundersen 1997). The schedule of the training camp was similar to that of the training at sea-level, and the training lasted approximately two weeks. The final phase was a testing week and included two, 5000 m time trials on the third and seventh days after return (Levine and Stray- Gundersen 1997). Plasma and blood volume were measured.

The results showed that there was no significant difference in sea-level training by any criteria

among the groups. However, plasma volume tended to increase in the high-low and high- high groups by training at sea-level at (Levine and Stray-Gundersen 1997). As hypothesized, plasma volume was unchanged in the sea-level control group throughout the study (Levine and Stray-Gundersen 1997). Living at moderate altitude, regardless of training altitude, resulted in a significant increase in red blood cell mass volume of 9% ($P < 0.01$), which was not observed in the control group (Levine and Stray-Gundersen 1997). In addition, after living at moderate altitude, plasma volume was shown to decrease, but this reduction was offset by an increase in red blood cell volume, leaving total blood volume unchanged, but with an increase in oxygen- carrying capacity (Levine and Stray-Gundersen 1997). Furthermore, in all three groups, training at sea- level improved 5000 m time trials, and after the training camp the 5000 m time was improved additionally, by an average of 13.4 ± 10 s for the high- low group ($P < 0.05$). The primary finding in this study was that acclimation to moderate altitude (2,500 m), when combined with training at low altitude (below 1,500 m), resulted in an improvement in sea-level running performance over 5000 m in already competitive athletes. Such an observation, however, was not seen when acclimatization was combined with training at moderate altitude or at sea-level (Levine and Stray-Gundersen 1997). Overall, it was shown that altitude acclimatization combined with training at low altitude produced a 1.4% improvement in sea-level performance (Levine and Stray-Gundersen 1997). Levine and Stray- Gundersen (1997) predicted that these physiological benefits of altitude training derived from either the development of acclimatization, enhancement of the training effect by hypoxic exercise, or both (Levine and Stray-Gundersen 1997). In regards to further research, it is still unclear as to why moderate altitude acclimatization only improves performance when training in low altitude and not moderate altitude.

Altitude improves sea-level performance in runners

Whereas Levine and Stray-Gundersen (1997) demonstrated that four weeks of acclimation to moderate altitude combined with training at low altitude was superior to an equivalent training camp at sea-level, Stray-Gundersen et. al. (2002) hypothesized that the combination of acclimation to 2,500 m and high-intensity training at 1,250 m would improve sea-level performance in elite middle- and long-distance runners.

In this study, 22 distance runners, 8 of which were women, were recruited. Athletes were required to be competitive at a national level (Stray-Gundersen et. al 2002). The study protocol was similar to that of the "living high- training low" study conducted in 1997. The major difference in this protocol was that athletes were required to perform high-intensity, high velocity training at 1250 m, and all other training took place at moderate altitude (5,000 m) (Stray-Gundersen et. al 2002). This modification is termed is termed the "HiHiLo" model. Sea- level performance was assessed by 3000 m time trial races on a 400 m all-weather track. Athletes were also asked to run on a treadmill with constant velocity and increase in grade of 2% every two minutes, until they experienced volitional exhaustion. Gas exchange, heart rate, and percent arterial oxyhemoglobin saturation were measured (Stray-Gundersen et. al 2002). Venous blood draws were also conducted three days before altitude exposure, nineteen days after altitude exposure, and twenty hours after return to sea-level. Maximal oxygen uptake (VO2max) was 3% higher after altitude camp. Hemoglobin concentration increased in acute ascent to altitude and remained elevated throughout camp, but then elevated on return to sea-level (Stray-Gundersen et. al 2002). In addition, plasma erythropoietin (EPO) concentration doubled after just one night at 2500 m, and decreased significantly after return to sea-level (Stray-Gundersen et. al 2002). After a twenty-seven day camp that utilized a HiLo paradigm, sea-level 3000 m running performance improved significantly. As Levine and Stray-Gundersen (1997) found, Stray-Gundersen et. al (2002) discovered similar results in that the runners improved sea-level performance by 1.1%. Stray- Gundersen et. al concluded that a stimulation of erythropoiesis leads to an apparent increase in oxygen delivery to tissues by a near doubling of plasma EPO concentration and an increase in hemoglobin concentration (Stray-Gundersen et. al 2002). Although this study seemed to answer the question of whether elite athletes could improve sea-level performance with altitude training, the question that arises is: what explains for the marked individual variability in response to altitude training? The implication with Stray-Gundersen et. al's (2002) study is that expansion of red blood cell mass and increased hemoglobin concentration at moderate altitudes contribute to improved sea-level performance.

Individual variation in the erythropoietic response to altitude training

Scientists such as Stray-Gundersen have worked on explaining the mechanism for an improvement in performance, but eventually were limited in their research because the proposed mechanism could not explain individual variability among athletes. Friedmann et. al (2005) examined the individual variation in the erythropoietic response to altitude training in elite junior swimmers. Great inter-individual variability has been found in the erythropoietic response to high and moderate altitudes (Friedmann et. al 2005). Friedmann et. al (2005) suggest that this variability might largely be explained by individual variation in the erythropoeitic response to altitude.

To test this hypothesis, EPO was measured in 16 elite junior swimmers, 11 of which were females, prior to and after four hours of exposure or normobaric hypoxia (sea-level) as well as repeatedly during living and training at an altitude of 2100-2300 m (Friedmann et. al 2005). Total hemoglobin mass was also determined with cardiac output (CO) rebreathing, before and after altitude training. Incremental swimming tests were also performed regularly (Friedmann et. al 2005). It was found that EPO was significantly higher after four hours of exposure to normobaric hypoxia by 58.3%, and on days one and two of altitude exposure by 51.8% (Friedmann et. al 2005). There was a large individual variation in the EPO response after four hours in normobaric hypoxia, as well as during altitude. A change in total hemoglobin mass showed a wide inter-individual variability. Overall, after three weeks, total hemoglobin mass was significantly increased by 6% on average (Friedmann et. al 2005). In addition, a considerable amount of individual variation was found in the improvement in maximal and submaximal performance. However, there was no significant correlation between the change in hemoglobin mass and the change in maximal performance. One explanation Friedmann et. al (2005) proposed for the unexpected lack of increase in total hemoglobin mass after altitude training was that there may be a substantial individual variation in the erythropoietic response to altitude which may have possibly been responsible for the lack of significant changes and might also explain the inter-individual variation in sea-level performance after altitude exposure (Friedmann et. al 2005).

This investigation confirmed the wide inter-individual variation in the erythropoietic response to altitude training in elite swimmers. It also demonstrated that the EPO increase which occurred during the first day of living and training at moderate altitudes correlated well with the EPO response after four hours of exposure equivalent to normobaric hypoxia. However, there is no simple linear relationship between EPO measurements during altitude training and the increase in total hemoglobin mass (Friedmann et. al 2005). This phenomenon may largely be explained by the presence of an inter-individual variation in response to EPO increase due to altitude (Friedmann et. al 2005).

Effects of high-altitude exercise training on cardiomyocytes

Another concern regarding altitude training is that of cardiac remodeling. Aerobic exercise not only induces cardiac morphological remodeling (increase in ventricular chamber dimensions and increase in myocardial mass) but also induces functional adaptations (Cozorla et. al 2006). Various cellular parameters have been examined to explain the beneficial effect of exercise on heart contraction. Training does not appear to modify intracellular Ca2+ transit. However, it increases Ca2+ sensitivity of the myofilaments and cardiomyocytes. Cazorla et. al (2006) designed a study to test the hypothesis that high altitude training limits the beneficial effects classically observed after training at sea-level. They investigated the effects of training under normoxic and hypobaric conditions on myofilament Ca2+ sensitivity and its stretch dependency in single skinned cardiomyocytes (Cozorla et. al 2006).

Male Wistar rats were randomly assigned to one of four groups (7 rats per group): rats living continuously either in normoxic conditions without or with aerobic training sessions (N and NT, respectively) and rats living continuously in hypoxic conditions with or without aerobictraining sessions (H and HT, respectively). High altitude was simulated, and rats were maintained for five weeks at different barometric pressures, and the training program consisted of five 45-minute sessions per week (Cozorla et. al 2006). Rats were anesthetized, and a two- dimensional short-axis view of the left ventricle was obtained. End-diastolic anterior and posterior wall thickness, and left ventricular end-diastolic and end-systolic diameters were measured, and heart mass was evaluated. The Ca2+ activated

force of single skinned myocytes was measured (Cozorla et. al 2006). In addition, sarcomere length (SL) was measured.

Efficiency of the training program in NT and HT rats was evaluated from maximal aerobic velocity and skeletal muscle citrate synthase activity (Cozorla et. al 2006). Both increased significantly after training, irrespective of environmental conditions. Left ventricular (LV) stroke volume increased significantly in NT rats, but remained unchanged in HT rats. Right ventricular (RV) mass increased significantly with hypoxic exposure, and LV mass increased significantly after exercise training was performed at either sea-level or high altitude (Cozorla et. al 2006). Sea level training improved the contractile properties of the myofilaments in the heart; this shows that there is an increase in contraction and Ca2+ sensitivity in cardiomyocytes (Cozorla et. al 2006). The Ca2+ sensitivity increased with training in normoxic conditions, but decreased when training was performed at hypoxic conditions. The stretch-dependent modulation of activation or Ca2+ sensitization is an important factor accounting for the Frank-Starling mechanism of the heart. It increased significantly with chronic exercise at sea-level, but depressed at high-altitude. This led to a reduction in this transmural gradient that may contribute to the lack of improvement in LV function via the Frank-Starling mechanism. Cazorla et. al (2006) propose that the enhanced LV stroke volume was due greatly to the ventricular filling associated with improved contractile properties of myofibrils; however, the final result was that high-altitude training depressed myofilament response to Ca2+ and thus, caused a lack of improvement in LV function (Cozorla et. al 2006).

Cardiac remodeling in rats

It has recently been suggested that a reduction in maximal stroke volume is an important mediator for the lower maximal cardiac output in acclimatized subjects after returning from altitude. Reboul et. al (2004) examine the effects of a five-week highly supervised altitude training camp on left ventricular (LV) and right ventricular (RV) morphologies and functions in sea-level native rats. Sixteen-week-old, sea-level-native Dark Agouti male rats were randomly assigned to live continuously in normoxia (N), living and training in normoxia (NT), living in hypoxia (2800 m) (CH), and living and training in hypoxia (CHT). Cardiac adaptations were evaluated throughout the study and maximal stroke volume (LVSVmax) was measured during volume overloading before and after the study period. RV free walls and LV plus septa were weighed (Reboul et. al 2004). Altitude training resulted in a specific LV remodeling compared with NT, characterized by an increase in wall thickness. At the end of the study period, altitude sea level training resulted in LV hypertrophy (Reboul et. al 2004). LV mass values in NT and CHT rats were statistically ($P < 0.05$) higher than those in the other two groups. On the other hand, RV mass was statistically ($P < 0.05$) lower in N compared with CH and CHT, and in NT compared with CHT (Reboul et. al 2004). Because both hypoxia and training affected RV mass, training at altitude induced a greater RV hypertrophy than living only at altitude. Before exposure, no significant differences were found between groups for all morphological data. Left mass (LVM) estimated by echocardiography was not modified until the end of the study period in N and CH rats, but significantly increased in NT and CHT rats. Moreover, the increase in

LVM was less pronounced in CHT than NT rats. Similarly, left ventricular stroke volume (LVSV) remained unchanged in N and CH rats, but progressively increased during sea level training in NT rats, values being significantly higher than those in the other three groups (Reboul et. al 2004). There was an observed decrease in LVSV in the two hypoxic groups during the first two weeks, which tended to increase two days after return from altitude. Furthermore, total hemoglobin concentration, as shown in previous studies mentioned in this review, significantly increased ($P <0.05$) in CH and CHT rats, but this trend was not seen in N and NT rats (Reboul et. al 2004).

The major result was the apparent limitation in LVSV improvement after training in CHT rats was mainly accounted for on the basis of affecting LV filling. Sea level training induced in NT an increase in LV internal cavity dimensions as well as wall thickness (Reboul et. al 2004). Different cardiac remodeling was observed in the CHT rats. This difference was characterized by an increase in a major LV wall thickness. Roboul et. al (2004) suggest that due to the already increased blood pressure during exposure to altitude, which is then exacerbated during exercise, a potential hypoxia-induced blood pressure increase could have been involved in the specific cardiac remodeling as shown in the CHT rats, by means of an increase in afterload, especially during exercise (Reboul et. al

2004). In summary, Reboul et. al (2004) showed a limitation of maximal heart pumping activity after altitude training compared with sea level training, explained by morphological and functional adaptations in the heart.

> Conclusion

In conclusion, altitude effects sea-level performance in athletes, and induces physical and physiological changes in the heart. In an early study Levine and Stray-Gundersen (1997) observed the effects on training of athletes who lived in altitudes above 2,500 m, but trained at altitudes below 1,500 m. The question that arose at the end of this study was why acclimatization to high altitude combined with training at low altitude was superior to the other groups that were tested. Stray-Gundersen et. al (2001) examined the effects of high altitude acclimatization and low altitude training on sea-level performance. Stray-Gundersen could not explain the huge amounts of variations in their study, but proposed the possibility of inter-individual variation in erythropoiesis. One major limitation to Stray-Gundersen et. al's (2001) research was the absence of a control group. The results found in this study could have been compared to a control group performing at a similar training camp at sea-level. This would have ensured that the athletes did not merely improve just from the training camp, but rather living high and training low. Friedmann et. al (2005) suggested that this was because there was high inter-individual variability in improvement in sea-level performance after training at moderate altitudes. In addition to improvement in sea-level performance after exposure to altitude, altitude also causes physical changes to the heart. Using a larger sample size and using different populations of people to determine whether genetics played a role in erythropoiesis in response to high altitude could further continue this research. Reboul et. al (2004) examined the effects of a five week highly supervised altitude training camp on left ventricular (LV) and right ventricular (RV) morphologies and functions in sea-level native rats. One thing that was not measured in this study was left ventricular stroke volume during maximal exercise. Furthermore, altitude also causes physiological changes in the heart. Cazorla et. al (2006) designed a study to test the hypothesis that high altitude training limits the beneficial effects classically observed after training at sea-level. They investigated the effects of training under normoxic and hypobaric conditions on myofilament Ca2+ sensitivity and its stretch dependency in single skinned cardiomyocytes (Cozorla et. al 2006).

Although most of this research was focused on aerobic capacity during exposure to altitude, it would also be important to study the anaerobic capacity during exercise at moderate altitudes. This could be done by using a modified version of the protocol used in Levine and Stray-Gundersen's (1997) study, however the anaerobic capacity would be measured and changes in lactic acid present would be recorded.

High altitudes stimulate the production of red blood cells, which causes an increase in oxygen carrying capacity, and thus, results in an improvement in sea-level performance. In addition to high altitudes effecting sea-level performance, it also causes physical and physiological changes in the heart, which could potentially further explain why sea-level performance is improved. This research is of great interest to many scientists as many athletes take advantage of enhancing erythropoiesis by training in high altitudes.

> References

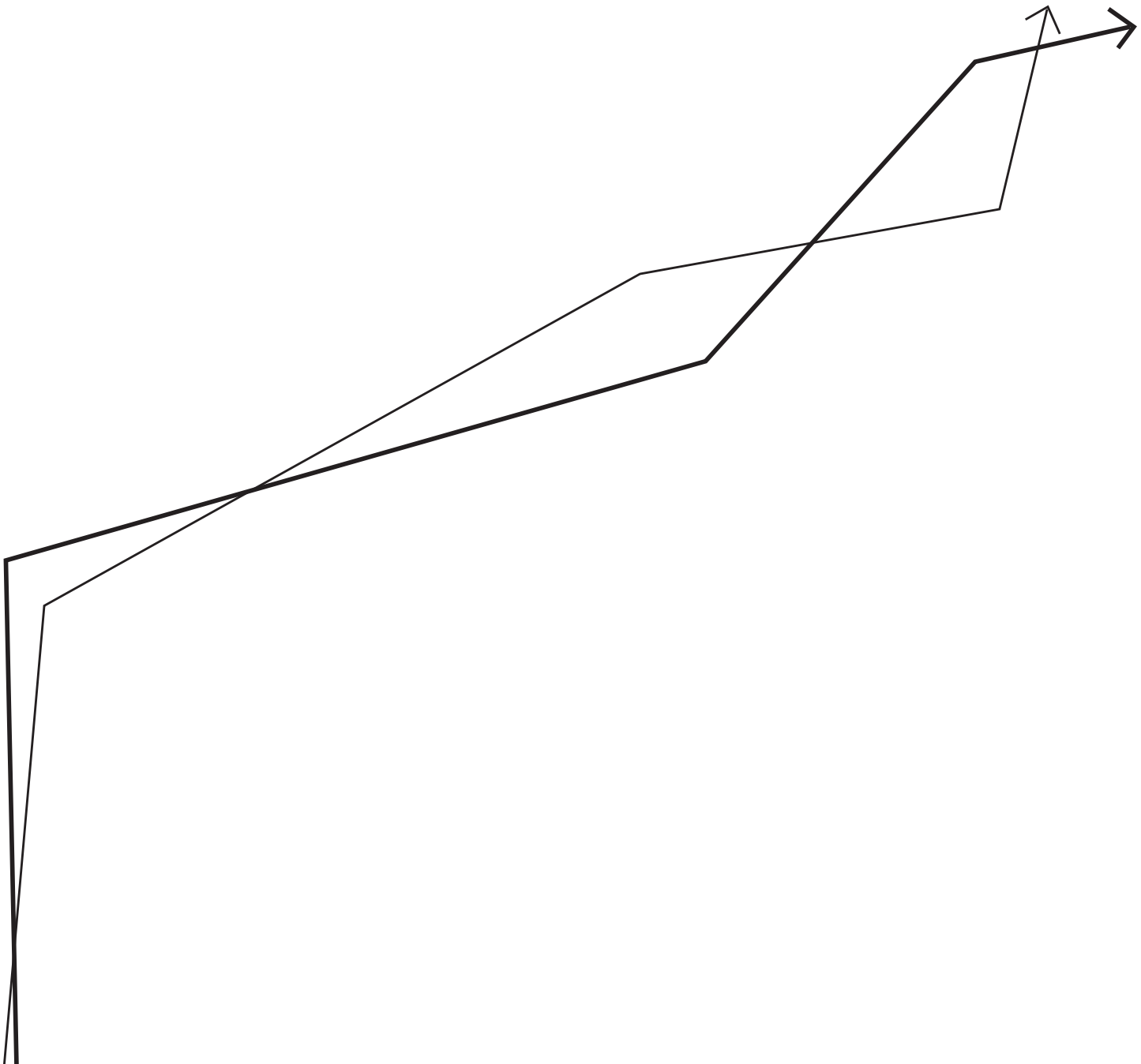
Cazorla O, Ait Mou Y, Goret L, Vossory G, Dauzat M, Lacampagne A, Tanguy S, Obert P. 2006. Effects of high altitude exercise training on contractile functions of rat skinned cadiomyocyte. Cardiovascular Research. 652-600.

Friedmann B, Frese F, Menold E, Kauper F, Jost J, Bartsch P. 2003. Individual variation in the erythropoietic response to altitude training in elite junior swimmers. British Journal of Sports Medicine. 39(3):148-153.

Levine, BD and Stray-Gundersen J. 1997. "Living high-training low": effect of moderate altitude acclimatization with low training on performance. American Physiological society: 102-111.

Reboul C, Tanguy S, Juan JM, Dauzat M, Obert P. 2004. Cardiac remodeling and functional adaptations consecutive to altitude training in rats: implications for sea level aerobic performance. J Appl Physio 98: 83-95.

Stray-Gundersen J, Chapman RF, Levine BD. 2002. "Living high-training low" altitude training improves sea level performance in male and female élite runners. Scand J Med Sci Sports 12(1):60




INK