
ERGO

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ACKNOWLEDGEMENTS

All articles in Ergo are peer reviewed by a committee of students and faculty from Weber State University. The staff and faculty advisers for Ergo would like to thank these students and faculty who volunteered their time and expertise reviewing manuscripts and contributing to the production of Ergo.

The success of this journal would not be possible without the commitment of everyone involved. We would also like to acknowledge Yaeko Bryner, Stephen & Susan Denkers Family Foundation Trust, George S. & Dolores Dore Eccles Foundation, Kem & Carolyn Gardner Foundation, Richard R., II & Karen W. Fairbanks, Ralph Nye Charitable Foundation, PSI CHI, and Gloria Z. Wurst for providing financial support for undergraduate research at Weber State University. This commitment to WSU students has provided unique and powerful learning opportunities that continue to impact students long after the projects are completed.

"The effort to understand the universe is one of the very few things that lifts human life a little above the level of farce, and gives it some of the grace of tragedy. "

-Steven Weinberg

European Budget Lodgings Attributes and Hedonic Prices

Author: Xin Zhang
Mentor: Cliff Nowell

Introduction

Tourism is growing fast and has become one of the essential sectors in the world economy since the 1990s. For supporting the fast growing tourism industry, bigger cities start to print maps indicating locations for hotels, restaurants, local attractions, and so on (Dennis 50). From this trend we can tell that accommodation plays an important role in travelling. In addition, youth and student travel have already become a major part in the global tourism market. These travelers are more independent and relaxed. Thus, they tend to choose more casual lodging when they travel (Benckendorff 13&24). Hostel is a type of lodging that satisfies their taste. Yet the other types of budget lodgings, such as guesthouses, as wonderful substitutions are also highly welcomed by travelers. Therefore, the focus of this paper is finding the answer for this question: "What change in budget lodging price per person per night is observable at the margin when the characteristics of budget lodging changes?"

According to Rosen's findings for hedonic pricing, budget lodging could be considered as an alternative that has many characteristics such as location of lodging, access to transportation, access to the internet, and other characteristics. Thus, in order to better answer the question for how the price varies according to the changes in amenities, we break down the topic interested into many sub topics for each characteristic. For instance, how much more travelers are willing to pay for budget lodgings that have their own restaurants than those that have none. After those sub topics are answered, travelers are able to find the best deal considering their individual preference for budget lodgings. The answers can also help lodging suppliers to make more rational decisions. In addition, we can also tell if the information about the lodgings is fully used by the travelers.

Literature Review

Hedonic pricing theory is used in this study to analyze the implicit market for characteristics of budget lodging in Europe. This theory suggested that price of a differentiated product can be interpreted as a function of its

characteristics, and each attribute has a price of its own in the market (Rosen 50). That idea is valuable for this study since, most of the time, consumers are only interested in some elements of the bundled attributes.

Recently, the hedonic method is used extensively for housing markets. Actually, the hedonic price theory has also been used on varieties of goods. In 2000, a study by Taylor and Smith indicated that the hedonic price model could be applied to rent rate for vacation amenities (Taylor 567). Also, there are many studies applying hedonic theory to products such as vegetables, wines, or lands.

Surprisingly, there is no empirical study applying hedonic price to analyze the budget lodging market, although the budget lodging market definitely shares certain traits with the housing market.

Fortunately, some empirical studies have tested the hedonic price theory by applying it to the hotel market, which is closely related to the budget lodging market. Espinet applied hedonic prices method to analyze holiday hotels in the sun-and-beach segment in Spain (Espinet 169). In 2010, Andersson did a research for hotel rooms in Singapore's market using hedonic price estimation. A mixture of log-linear and semi-logarithmic function forms are used in regression analyzing (Andersson 232). In the same year, Kuminoff, Zhang, and Rudi investigated consumer's willingness to pay a premium for environmentally friendly hotels. That study mentions some difficulties faced by applying hedonic price theory, and it started by a linear hedonic price function for the modeling part and then used the results to serve the meta-analysis which followed (Kuminoff 478). All three of those studies indicate that hedonic price functions could be applied to the budget lodging market, which is very similar to the hotel market, and two of them also provide possible functional forms for hedonic regressions.

Theoretical Model

In this study, the theoretical model is based on hedonic price estimation. In order to build up the theory, it is better to start with the simplest structure that assumes each budget lodging with only one attribute. Take level of security as an example; let it be denoted as variable z. Within a budget lodging market, the price of budget lodging can be expressed as a function of level of security. In other words, the price of budget lodging can be expressed as p(z).

Consumer

Consumers, who pay for both budget lodging and other goods, face the problem of how to maximize their utility. Assume that a consumer has utility function U and income level of y. In order to maximize utility, the decision

of how to allocate the income between budget lodging and other goods is essential. Denote the amount of income spent on other goods as *x*, and then the problem this consumer has can be expressed mathematically as the following:

$$Max\ U(x,z)$$

with budget constrain of:

$$y = x + p(z)$$

Now, if the level of *z* is fixed, the level of *x* can be determined for some fixed amount of utility $U(x,z) = \bar{U}$. After the level of *x* is known, the income available for purchasing budget lodging can be determined as $\theta = y - x$ with $\bar{U}$ level of utility. Therefore, the consumer's problem can also be defined, given values of *z*, *y*, and $\bar{U}$, as a bid function $\theta(y, z, \bar{U})$ which represents the maximum amount this consumer is willing to pay for the budget lodging with attribute *z* (Rosen 39). For an individual consumer to reach the equilibrium, the marginal willingness to pay has to equal to the marginal implicit price of the attribute *z* (Freeman 372).

Producer

While consumers seek for maximum utility, producers focus on maximizing their profit. Assuming constant return to scale, set the factor prices as ß, so that the cost for per unit of budget lodging is going to be c(ß,z). The profit that seller tries to maximize is going to be:

$$\prod = \phi - c(ß,z)$$

An offer function that indicates at what price the seller is going to sell the product to earn a certain given level of profit , for this seller can be expressed as $\phi(ß,z,)$ with given level of factor price and attribute z. The offer function indicates the minimum price a seller is willing to accept as a function of c(ß,z) and attribute z. Again, as a price taker, the seller can only make the deal when the offer function touches the hedonic price line (Rosen 41-43).

Market Equilibrium

The consumer's choice and the seller's choice were discussed, and now the next step is to talk about the hedonic price function itself. For the equilibrium to occur, the bid function and the offer function must touch the hedonic price function (Freeman 127). Alternatively, it means that their slopes are all the same. Notice that the slope of hedonic function curve is also

the marginal price. Finally, the slope of bid function indicates how much more the consumer is willing to pay for one more unit of attribute z (Rosen 38, 44).

Rosen's findings

So far the hedonic price theory is analyzed under the assumption that the budget lodging has only one attribute. In reality, the product usually has more than one characteristic. With multiple attributes, the major extensions from the theory developed above are two. First, the p'(z) should be replaced by $p'(z_i)$ since the price of products can be expressed as a function of many attributes $z_1, z_2, z_3, \ldots z_n$, with i=1,2,3,...n. But the meaning of $p'(z_i)$ is, similar to p'(z), marginal price which illustrates how much changes per unit change in the will bring to price. Second, instead of only one market, n markets for n product characteristics are considered simultaneously. They form a 2n simultaneous equation system where equilibrium can be obtained only if the simultaneous solution to these equations is found.

Data and Selected Variables

Population

According to Rosen, hedonic price works under an assumption of competitive market. In this study, the market is defined as the budget lodging market in some regions in Europe. Those specific regions are chosen according to the rule that the market in those regions could be assumed as a competitive market (Carlton 57). Overall, 1438 budget lodgings are included in the population. This study uses data from an online travel agency, HostelBookers, as its source of empirical observations.

Sampling and Variables

A total of 103 budget accommodations in the population were randomly sampled. Price data and attribute data were collected for each accommodation. Prices were per person per night and were expressed in US dollars. Since the price for those budget lodgings does not change with time, the price data at November 13, 2011, was collected. The number of attributes is too large for any manageable statistical model; therefore, the most relevant ones were chosen by giving priority to:

I. The attributes appearing in the online travel agency HostelBookers. Assuming rational marketing management, attributes that are displayed there should be those most valued by customers.

II. The availability of information for all the budget lodgings in the sample (Espinet 167&168).

III. Characteristics that show statistical significance in exploratory analyses carried out with regression models.

Empirical Model

In this study, the challenge of using hedonic price estimation is estimating the correct hedonic price function econometrically. After applying the Box-Cox transformations, the resulting λ value suggests that the semi-logarithmic functional form is the best fit for this study.

Model 1

$$\begin{aligned}\text{Ln Price}_{ijk} = {} & \alpha_0 + \alpha_1 \text{PSinE}_i + \alpha_2 \text{PSin}_i + \alpha_3 \text{PTwiE}_i + \alpha_4 \text{PTwi}_i + \alpha_5 \text{PDouE}_i \\ & + \alpha_6 \text{PDou}_i + \alpha_7 \text{PTriE}_i + \alpha_8 \text{Apt1}_i + \alpha_9 \text{Apt2}_i + \alpha_{10} \text{StdApt}_i \\ & + \alpha_{11} \text{Internet}_i + \alpha_{12} \text{24Recp}_i + \alpha_{13} \text{WashM}_i + \alpha_{14} \text{DistanceTra}_i \\ & + \alpha_{15} \text{Star3}_i + \alpha_{16} \text{Star3.5}_i + \alpha_{17} \text{Star4}_i + \sum_{j=18}^{25} \alpha_j \text{ CityName}_j \\ & + \sum_{k=26}^{30} \alpha_k \text{OtherAttri}_k + \varepsilon_{ijk}\end{aligned}$$

Model 1 suggests that the influence of DistanceTra on natural log of price is linear. That might be true within short distance. However, situations might differ for longer distance. In order to figure out the influence of longer distance on price, the squared DistanceTra term is added to Model 1 to get the following Model 2 (Gujarati 502).

Model 2

$$\begin{aligned}\text{Ln Price}_{ijk} = {} & \beta_0 + \beta_1 \text{PSinE}_i + \beta_2 \text{PSin}_i + \beta_3 \text{PTwiE}_i + \beta_4 \text{PTwi}_i + \beta_5 \text{PDouE}_i \\ & + \beta_6 \text{PDou}_i + \beta_7 \text{PTriE}_i + \beta_8 \text{Apt1}_i + \beta_9 \text{Apt2}_i + \beta_{10} \text{StdApt}_i \\ & + \beta_{11} \text{Internet}_i + \beta_{12} \text{24Recp}_i + \beta_{13} \text{WashM}_i + \beta_{14} \text{DistanceTra}_i \\ & + \beta_{15} \text{Star3}_i + \beta_{16} \text{Star3.5}_i + \beta_{17} \text{Star4}_i + \beta_{18} \text{DisTra} * \text{DisTra}_i \\ & + \sum_{j=19}^{26} \beta_j \text{ CityName}_j + \sum_{k=27}^{31} \beta_k \text{OtherAttri}_k + \mu_{ijk}\end{aligned}$$

Since this study deals with a cross-section data set, there is a good chance that this model suffers from heteroscedasticity (Gujarati 503). Since the sample size is sufficiently large, graphical method is applied for detecting heteroscedasticity. The resulting plots for both models indicate that there are probably no heteroscedasticity presenting in the data.

Empirical Results

Table 1. Results from OLS regression

	Model 1		Model 2	
Predictor	Coefficient	Standard Errors	Coefficient	Standard Errors
Dublin	-0.276***	0.066	-0.254***	0.065
Krakow	-0.418***	0.058	-0.419***	0.057
Berlin	-0.116**	0.058	-0.103*	0.057
Prague	-0.473***	0.062	-0.532***	0.062
Belgrade	-0.456***	0.082	-0.456***	0.081
Lisbon	-0.382***	0.060	-0.375***	0.059
Athens	-0.431***	0.069	-0.460***	0.068
Budapest	-0.467***	0.057	-0.444***	0.056
PSinE	0.872***	0.064	0.858***	0.063
PSin	0.811***	0.071	0.805***	0.069
PTwiE	0.372***	0.067	0.348***	0.066
PTwi	0.336***	0.051	0.332***	0.050
PDouE	0.408***	0.051	0.395***	0.050
PDou	0.360***	0.050	0.363***	0.049
PTriE	0.215***	0.054	0.204***	0.053
Apt1	0.468***	0.096	0.492***	0.095
Apt2	0.454***	0.098	0.493***	0.096
StdApt	0.329***	0.122	0.340***	0.119
ACre	-0.051	0.036	-0.037	0.035
24Recp	-0.088**	0.036	-0.099***	0.036
LugRoom	-0.013	0.037	-0.009	0.036
Internet	0.133**	0.056	0.108*	0.056
WashM	-0.154***	0.035	-0.156***	0.034
Restaurant	0.069	0.045	0.010**	0.045
DistanceTra	-0.000098**	4.336E-05	0.000366***	0.0001268
Star3	0.176***	0.050	0.165***	0.049
Star3.5	0.282***	0.093	0.265***	0.091
Star4	0.697***	0.102	0.738***	0.100

Table 1. Continued

	Model 1		Model 2	
Predictor	Coefficient	Standard Errors	Coefficient	Standard Errors
CurrencyEx	-0.061	0.046	-0.064	0.045
Beach	-0.021	0.062	-0.015	0.060
DisTra*DisTra			-0.00000034***	9E-08
Constent	3.475***	0.066	3.424***	0.06558
F	31.12***		31.84***	
R^2	73.2%		74.30%	
$\overline{R}^2$	70.8%		72.00%	

*Note: Data source is http://www.hostelbookers.com, and Google Maps is used for collecting distance data. N=372. *,**,*** represent significant result at 0.1, 0.05, and 0.01 confidence levels, respectively.*

Conclusion

In this study, the methodology is carried out for budget lodgings in thirteen European cities. The results show that cities in which budget lodgings are located will significantly affect consumers' willingness to pay. Comparing with shared rooms, private rooms are more expensive. However, this increase in price brought by being a private room disappears when the capacity of people increases in private rooms. When the capacity for private room reaches three persons, with no individual bathroom, the private room does not differ from shared rooms for consumers. It shows that privacy is valuable for travelers. Hotels are, as expected, more expensive than other types of lodgings. But per person rate for 1-star and 2-star hotels results in no difference to other types of lodgings such as hostels. It is surprising to find out that distance to the city center does not affect price significantly, no matter if the measurement is in driving time or walking distance. The convenient public transportation system in Europe might be one possible explanation for this finding. It is interesting to find out that disclosing some attributes, such as WashM, for the lodging reduces consumers' willingness to pay because these attributes might reduce consumers' expectation for the lodging.

The insignificance of many facilities and service attributes shows that price of budget lodging is mainly determined by the very essential function for lodgings, or consumers only concern about basic lodging requirements. Or, travelers do not actually consider all of the attributes in the data collected in this study when they decide where to stay (Kuminoff 477). There are incentives for travelers to gather information about budget lodging and make

wise purchase decisions. Disclosure of characteristics is essential for that process, yet disclosure of information is useful only if travelers do use the information (Stanley 539-540). The results in this study illustrate that travelers might not respond to all the disclosure attributes.

Further Study

Another direction for further research would be to apply multilevel models for the estimated hedonic price functions. These hedonic functional forms are carried out in many previous studies for housing markets and some studies for hotel markets (Espinet 169). Moreover, the information provided by the data source used in this study is not perfect. For further research, a more detailed and careful primary data-collecting is needed.

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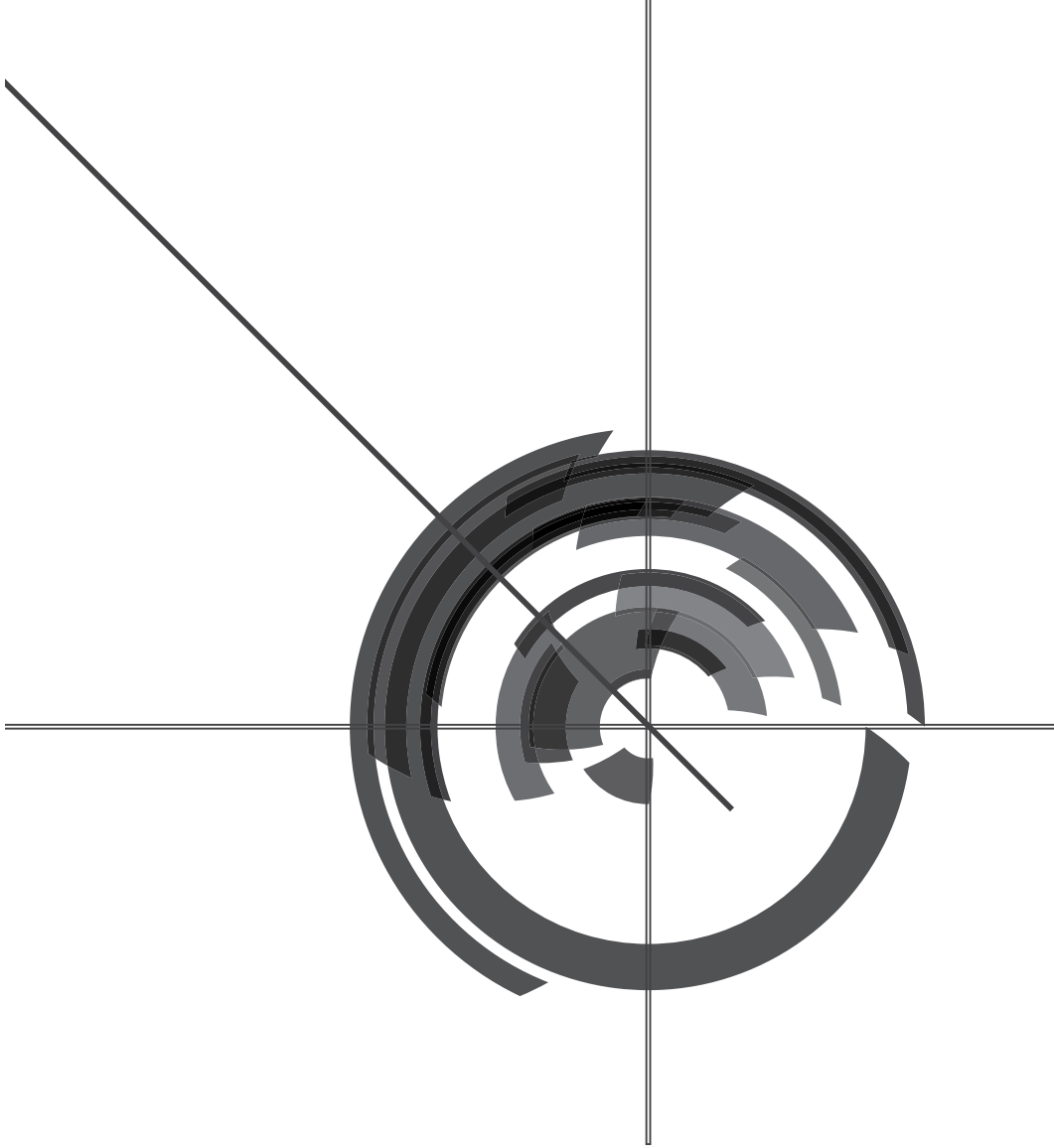


Has the Adoption of Home Value Code of Conduct Negatively Impacted the Value of Homes?

Author: Jeremy Scott Tricozzi
Mentor: Dorris Geide-Stevenson

Abstract: This paper argues that the industry wide adoption of the home value code of conduct and use of appraisal management companies has led to a downward influence on home prices. An ordinary least squares regression, implementing the change of rules as a dummy variable, reveals that there was a statistically significant impact on the price of homes due to this rule change.

Introduction

The purpose of this research paper is to test whether the way in which appraisers are hired has a statistically significant relationship on housing prices. Section II describes the change in how appraisers are hired. Section III develops the equilibrium model based on supply and demand functions. An explanation of the data that was used can be found in section IV. Section V will develop an empirical model to test the statistical significance of the rule change on housing prices. Section VI reviews the results and section VII concludes.

Background

In 2007 in the state of New York, then Attorney General, Andrew Cuomo subpoenaed the nation's two largest mortgage financiers, Fannie Mae and Freddie Mac. Instead of releasing its data on appraisals, Fannie and Freddie struck a deal with Cuomo. Part of this deal included the adoption of new rules called the Home Valuation Code of Conduct (HVCC). Primarily HVCC changes the way in which appraisers are hired (Biggers 2010). Under these new guidelines, mortgage brokers were no longer allowed to directly hire anyone to perform an appraisal. Brokers must now hire appraisers from a lenders approved list. Most lenders are large and they rely on the use of Appraisal Management Companies or AMCs. With this change in the rules and the appraisal industry itself, it is expected that the bias on home prices has altered. This leads to the research question; "Did the adoption of HVCC rules negatively impact the value of homes in the U.S.?"

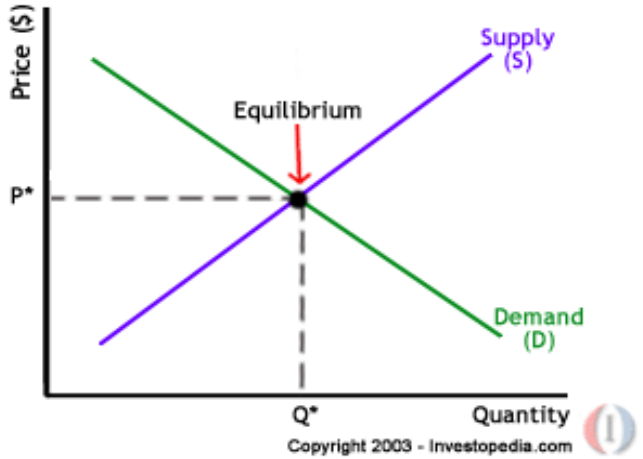
Modeling and a Review of the Literature

A.Equilibrium Model

This research paper evaluates a change in an industry rule that affected the entire U.S. mortgage market. The sales price or equilibrium price of homes can be estimated with supply and demand equations.

1. Price as a function of supply and demand

Evans (1995) establishes a basic demand and supply model of the housing market and identifies major factors that affect equilibrium home prices (P*). P* is determined at the intersection of the supply and demand curves in Figure 2.



2. Demand Function

Quigley(1999), offers a basic demand function adding aggregate employment, population, and income to the demand model for housing. In a comparison of regional home prices to national home prices, Alan Reichert(1990) develops a more expansive demand model. He adds quality of new homes produced, the real interest mortgage rate, loan to value ratio, speculation and seasonality to the demand equation (1).

$$Hd_t = Hd_t\,(\,POP_t, INC_t, EMP_t, RATE_t, LtV_t, NHQ_t, SPEC_t, SEASON_t)$$

Where Hd_t represents housing demand at time t, NHQ_t is the quality of new homes, POP_t is the population INC_t is the disposable income, EMP_t is the employment level, LtV_t is the loan to value ratio, $RATE_t$ is the closing mortgage rate, $SPEC_t$ is a measure of speculative investment and $SEASON_t$ represents the four seasons of the year.

3. Supply Function

Housing capital costs, the price of housing and the mortgage rate have been commonly used factors that determine housing supply. (Potepan 1996, Reichert 1990, Quigley 1999, Malpezzi 1996). Housing is analogous to other products, in that its supply is governed by production. The production of housing is influenced by the markets of home construction inputs. The cost of building a home can be broken down into the cost of its materials, mainly lumber, cement, steel, labor and the cost of the land the home is built on. Profits rise as the gap between costs and price widen and there is an expected increase in the amount of new homes produced (Reichert 1990). Investing into home building is very speculative because of the large length of time between the planning and sale of a home. Reichert (1990) believed the accelerated increase in new home prices would stimulate speculative investment. So a measure of the acceleration in new home values will be added to the supply equation (Reichert 1990). The construction of homes can also vary by the season. (Carliner 2002). By combining these variables into a supply equation it is theorized that:

$$HS_t = HS_t\,(CONSTC_t, SPEC_t, SEASON_t)$$

Where HS_t represents housing supply at time t, $CONSTC_t$ is the combination of the all the costs that go into the construction of a home, $SPEC_t$ is a measure of speculative investment and $SEASON_t$ is a representation of the four seasons of the year.

Once the supply and demand functions are combined, a measure for rule change can be added. Malpezzi(1996), estimates the effect of several regulations on different metropolitan areas. Following Malpezzi's approach, the HVCC rules will be added to the model as a dummy variable.

4. Equilibrium Price

By combining the supply and demand equations in (1) and (2) as well as adding rule change, the following formula to estimate the equilibrium price of housing is created:

$$Ph_t = Ph_t\,(POP_t, INC_t, EMP_t, LtV_t, RATE_t, NHQ_t, CONSTC_t, SPEC_t, SEASON_t, RULE_t)$$

Where Ph_t represents the price of housing at time t, $RULE_t$ is the dummy variable used to represent a rule change at time t and all other variables retain their previously described meaning.

IV. Data

Home Prices: The price of homes is used as the dependent variable. Standard and Poor's has several home price indices based on repeat home sales. A quarterly U.S. composite index was chosen.

Population: Population is a measure of the total number of people living in the United States. It is expected that there is a positive relationship between population ***POP*** and home prices ***Ph.*** Data on population was gathered from the National Census website (U.S. Census Bureau 2011).

Income: Income is the amount of disposable income that U.S. citizens have available at time t. I would expect income ***INC*** to have a positive relationship with home prices ***Ph***. A measure of total national disposable income found on the Federal Reserve Bank of St Louis's website has been chosen to represent income.

Employment Rate: Employment measures the percentage of employable citizens in the U.S. who currently have a job. Given a higher employment rate, a greater percentage of the population is working, increasing demand for housing. This will generate a positive relationship between the employment rate ***EMP*** and home prices ***Ph***. Bureau of Labor Statistics provided the necessary monthly employment rate.

Loan to Value: The loan to value rate is a measure of the amount of total home value financed by the lender. As this rate increases it becomes easier for home buyers to purchase a home due to the fact that they will have a lower down payment. It is expected that the relationship between loan to value ***LtV*** and home prices ***Ph*** will be positive. Data on monthly loan to value rates was gathered from the Federal Housing Finance Agency website.

Contract Rate: When a loan is finalized the rate on the loan is the closing or contract rate (Perkins 2011). Data on the closing rates of individual mortgages were available through the Federal Housing Finance Agency website. Lower mortgage rates cause the monthly cost of owning a home to decrease. It is therefore anticipated that a negative relationship will exist

between home prices ***Ph*** and the contract rate ***RATE***.

New Housing Quality: New housing quality measures the quality of newly constructed homes in comparison to existing homes on the market. The quality of new homes is derived by dividing the monthly average price of new homes by the monthly average price of existing homes (Reichert 1990). If the quality of new homes increases it is likely that the effect of ***NHQ*** on home prices ***Ph*** will be positive. The monthly averages for new homes sold on the market were available on the National Census website and the National Association of Realtors.

Construction Costs: This is an average of cost indices for the costs that are involved in constructing new single family homes. Labor costs were calculated by taking the average wage of a construction worker between 2000 and 2011. Using the year 2000 as a base year an index for labor costs was constructed. The index for lumber, cement, steel and labor were averaged together into a final index of construction costs . An increase in this index would mean that the price to build a home has increased. It is theorized that a positive relationship between construction costs ***CONSTC*** and home prices ***Ph*** exists. The lumber, cement and steel indices were taken from the International Monetary Fund Index webpage, and wages for construction workers were available from the Bureau of Labor Statistics.

Speculation: A percent change in new home prices between each time period will be calculated. This percentage change will then be lagged by a one year period. With the expectation that the future values of homes will increase, it is expected that the relationship between speculation ***SPEC*** and home prices ***Ph*** will be positive. Data for new home prices and existing home sales prices were available through the Census and National Association of Realtors, respectively.

Seasonality: Is a set of dummy variables to account for the four seasons of the year. The relationship between home prices ***Ph*** and ***Q2, Q3*** is expected to be positive. ***Q4*** may have a negative relationship with home prices due to lower home construction during winter months.

Rule Change: Setting up a dummy variable will account for the rule change that occurred. The expectation is that there is a negative relationship between Rule Change ***RULE*** and Home prices ***Ph.***

V. Empirical Model

The use of an ordinary least squares regression will be implemented to discover the significance of the rule change on the dependent variables holding all other independent variables constant. The formula to estimate the equilibrium price of housing states that the price of housing is a linear additive function of population, income, employment, loan to value ratios, the closing rate, a measure of new home quality, construction costs, speculation, seasonality and rule change. Where the price of housing is greater than or equal to zero. The variable construction costs ***CONSTC***, is a measure of the costs associated to the construction of new homes and has no bearing on the cost of existing homes. To adjust the model, a new variable will be created. Dividing the construction costs of building new homes ***CONSTC***, by the quality of new homes ***NHQ***, the following new variable is derived ***CONSTC/NHQ***. Incorporating the new variable yields the following equation;

$$Ph_t = b_0 + b_1 POP_t + b_2 INC_t + b_3 EMP_t + b_4 LtV_t - b_5 RATE_t - b_6 CONSTC_t / NHQ_t + b_7 SPEC_t + b_8 Q2 + b_9 Q3 - b_{10} Q4 - b_{11} RULE_t$$

Where b_0 is the intercept and b_1 through b_{11} are the relative regression coefficients, or betas, for each respective variable. ***Q2, Q3*** and ***Q4*** are dummy variables for the respective months or quarters to account for seasonality at the given time period t. The null hypothesis is that b11 is zero, that the rule change had no impact on housing prices, all other factors constant.

VI. Results

Variable		US Composite
33Population	+	0.00002174*** (5.48)
Income	+	-0.033178*** (-4.39)
Employment	+	32.055*** (5.19)
Loan to Value	+	-3.083 (-1.58)
Contract Rate	–	-8.338 (-1.07)
Constc/NHQ	+	-0.2574 (-0.28)
Speculation	+	0.523 (0.38)
Q2	+	3.970 (0.51)
Q3	+	4.365 (0.48)
Q4	–	-5.76 (-0.54)
Rule Change	–	-34.71** (-2.25)
R-squared		86.5%
R-Squared(adj)		81.0%
F-Test Stat.		15.75

In the final regressions the null hypothesis was rejected within a 95% confidence interval. It can then be said that there is a statistically significant negative relationship between the home prices and the HVCC rules. The model also had a high R-squared value and F-test statistic, proving that the model predicted the price of housing well. Income should have shown a strong positive relationship with the dependant variables. Instead it showed a statistically significant negative relationship. After testing income with the other variables in the model it was found that income and population suffered from multicolinearity. Since income is the most important determinate in purchasing a home it was kept in the final.

VII. Conclusion

A model was developed to test the statistical significance of the HVCC rules and widespread use of AMCs on multiple measures representing the price of homes in the US. The results consistently showed a strong negative correlation between this rule change and housing prices. A new weighted average of housing prices was developed and calculated using current data. A new construction cost index was also formulated using current data. Finally the theoretical model developed was tested and corrected for autocorrelation.

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How Much Water Does the Great Salt Lake Need?

Author: Nathan Waugh
Mentor: Daniel Bedford

Abstract: For thousands of years, the Great Salt Lake has been an ecological cornerstone of the desert American west. As the largest water reservoir between the Mississippi River and the Pacific Ocean, past changes in the lake's water level and salinity have strongly impacted surrounding populations of humans and wildlife alike, with the lake's variable nature historically playing an important role in human settlement decisions. More recently, the lake has become an economic cornerstone as well, thanks in large part to Utah's active brine shrimp harvesting industry. As climate and population patterns in the Wasatch Valley continue to change, the lake's governing variable parameters must be examined in detail, with an eye to alleviating extreme fluctuations, so that the lake can continue in its important ecological and economic roles.

Background

Given the recent and projected human population expansion of the Wasatch Valley, it is important that both policymakers and citizens understand the impact of their actions on the Great Salt Lake (GSL) basin system. To that end, this paper will explore the history of fresh water fluctuation within that system, as well as the ways in which this variation has historically affected basin life and its implications for future development and use of the GSL water basin system. This paper will finish by looking at possible water use guidelines which might indefinitely keep the GSL basin a viable center of civilization, commerce, and unique natural habitat.

To understand the importance of fresh water to biological activity in the GSL basin, one must first understand the physical system of the basin itself. GSL is a terminal lake situated deep within the semi-arid desert of central North America. As such, it lacks any outflow to the ocean and is thus capable of losing water only through evaporation, a process which over geological time scales leaves behind enormous quantities of minerals and salts. In its multi-millennial existence, the lake has accumulated vast deposits of such salts, mainly sodium chloride, but also significant amounts of potassium, magnesium, and sulfate (Gwynn, 2002). While high doses of these substances are toxic to many forms of life, the lake-basin system is more biologically viable than one might at first assume. This is because the relative amount of dissolved salts present in the lake at any given time is highly dependent on both the physical location under consideration within the lake itself, as well as the influx of fresh water received by the lake annually from precipitation and snow melt.

GSL possesses several internal man-made, physical boundaries which inhibit water flow and salt exchange, by far the most impactful of which is the east-west Southern Pacific Transportation Company railroad causeway. Built in 1959, this causeway bisects the entire lake into two separate bays: Gunnison Bay in the north, and Gilbert Bay in the south. Each of GSL's three primary fresh water sources—the Jordan, Bear and Weber Rivers—empty directly into Gilbert Bay, while Gunnison Bay is left without any such constant fresh water source.

The result of this differential input is that the salinities of the two halves of the lake have diverged radically over the last 50 years. Though the entire lake once maintained a mostly uniform salinity, which varied between 15 and 27 percent, Gunnison Bay has since 1959 maintained an almost constant 28 percent salinity. Gilbert Bay, on the other hand, has gradually dropped in salinity until the 1970s, and has since held mostly to a much narrower salinity range of 6 to 15 percent (Stephens, 1998).

Once this salinity difference was established, the lake ecosystem also diverged. In Gunnison Bay, where fresh water input is lacking, few macro-organisms now live, and fewer still can thrive. This is due both to the increased osmotic stresses on these organisms as well as to the decreased oxygen content of the high-salinity water. Gilbert Bay, meanwhile, consistently maintains an ecosystem which more closely resembles that of entire lake prior to 1959, with greater levels of biological diversity and sustainability than can be found in Gunnison (Stephens, 1998). These differences perfectly illustrate the two important inverse relationships which the Great Salt Lake maintains with regard to salinity—one between lake level and salinity, and the other between salinity and biological diversity. It can thus be shown that the higher lake levels which result from increased fresh water input to the Great Salt Lake correlate with greater biological viability.

Though detailed records of lake levels—and, through them, indirect markers of total fresh water inflow—have been kept only since 1875, in the last 136 years the lake level has displayed a tremendous range of variability. At its lowest point, in 1963, the average lake level stood at approximately 1257 meters above sea level, while at its highest point, in 1986-87, the lake rose by an additional 7 meters. Because GSL sits in a gradually sloping desert basin, this seven-meter difference in lake level was sufficient to increase lake surface area by more than 140%, resulting in the flooding of large tracts of heavily developed land and causing direct property damages in excess of $500 million (adjusted for dollar inflation from 1985 to 2011) (Bedford, 2005).

GSL flooding not only damaged human structures, but changed the ecosystem of the lake in a manner comparable to the causeway installation of 1959. When the lake is at or near its historic average height of 1210 meters above sea level, biodiversity is limited primarily to bacteria, brine flies, brine shrimp, lake birds, and several species of protozoa. During the 1980s flood, however, biodiversity exploded with the introduction of several species of zooplankton and even a type of fish (Stephens, 1998). Perhaps the most significant of these low-salinity newcomers was the corixid *Trichocorixa verticalis*, which preyed heavily on brine shrimp population while the lake remained high. The brine shrimp predation was strong enough that commercial harvesting of brine shrimp cysts ground to a halt, significantly interfering with a dominant northern Utah commercial industry (Wurtsbaugh & Berry, 1990).

We can thus see that large fluctuations in lake salinity due to variations in lake level can have ill effects on the natural ecology and human economy of the lake-basin system. This is true regardless of whether the lake is rising or falling. It is uncertain to what degree these ill effects would hold if surrounding human civilization and lakeshore biology had a chance to adapt to long-term low or high salinities. Perhaps a clue can be found in the historical record of human and animal lakeside habitation.

The first humans to arrive on the shores of the GSL appeared more than ten thousand years ago. These prehistoric Native American tribes lived a hunter-gatherer lifestyle, subsisting on the vast caloric resources provided by the wetlands which surround the lake. These wetland resources were richest at those points where the brackish lake waters were diluted and enriched by the freshwater inflow of the major rivers and networks of streams that exist predominantly along the east and northeast borders of the lake. Among the animal and plant foods available for consumption in these wetlands were large populations of fish, bison, antelope, deer, waterfowl, and several varieties of plant life. Food levels varied by season and year, but given that the archaeological evidence for human lakeside settlement extends as far back as ten millennia ago, we can infer that for the majority of the lake's existence it has generated enough caloric richness to sustain human populations. Indeed, after the advent of lakeshore farming two thousand years ago, during what is now termed the Fremont period, human populations near the lake and surrounding wetlands grew significantly. What is more, these societies grew up precisely at those points along the lake where centers of human civilization currently exist (Simms & Stuart, 2002).

This growth in population, which brought with it a commensurate increase in variability between foraging and farming lifestyles, was only possible while sufficient water existed to maintain crops and natural wetland resources. Near the end of the late Fremont period, the GSL region began to

experience long-lasting and severe droughts which proliferated throughout the 12th and 13th centuries. These droughts negatively impacted the output of farms as well as the amount of biota available to foragers in nearby wetlands. This crowding of resources in turn made further human expansion impossible without risking inter-tribal conflict. As a result of all these factors, Fremont-era lakeshore farming declined, and many human populations left the GSL basin system entirely (Simms & Stuart, 2002).

Given such evidence for the effects of lake level and salinity on nearby human, animal and plant populations, it can be clearly seen that the viability of human population centers situated in the GSL basin are highly sensitive to the basin's various fresh water inputs. While these inputs do not depend entirely on lake-generated rainfall or snowpack melt, nearly 60 percent of the fresh water received by the Great Salt Lake comes from the Bear, Weber, and Jordan rivers (Bedford, 2005). Thus, river inputs must be a primary concern if we are to ensure that lake resupply and water balance ratios remain at optimal levels. Before this can be done, however, it must be determined what salinity and lake-level parameters are optimal, both to the human population around the lake and to the underlying ecosystem on which that population depends.

Proposal

One way to approach the question of optimal parameters is to look first at ecosystem effects on human economies. Because the varieties and amounts of all commercially utilized biota present in the lake and surrounding wetlands are sensitive to changes in lake salinity, and because brine shrimp harvesting is the dominant biota-dependent industry in the lake-basin system, the lower limit on fresh water input from the Bear, Jordan, and Weber rivers can safely be defined as that range wherein fresh water input remains high enough—and thus lake salinity levels low enough—for brine shrimp reproduction to take place. Similarly, the maximum upper bound on fresh water input can be defined as that level of water input which decreases lake salinity sufficiently that *T. verticalis* can easily reproduce and damage brine shrimp populations (Stephens, 1998).

Maintaining the lake within these parameters would involve planning and policy decisions beyond the scope of this paper. However, one thing that every person, business, and organization can do to ensure the viability of the GSL basin as a population center is to conscientiously examine and adjust water usage habits. For instance, it is fairly common in Utah to see lawn sprinklers activated in the middle of the day, when most of the water is lost to evaporation, or in the middle of a rainstorm, when it is not needed. This is of particular importance because lake water shortages tend to be

more common than do water surpluses. Thus, careful monitoring of wasteful water practices might go a long way toward relieving the burden of fresh water input that the GSL increasingly faces, leaving its shores a better habitat for person and animal alike.

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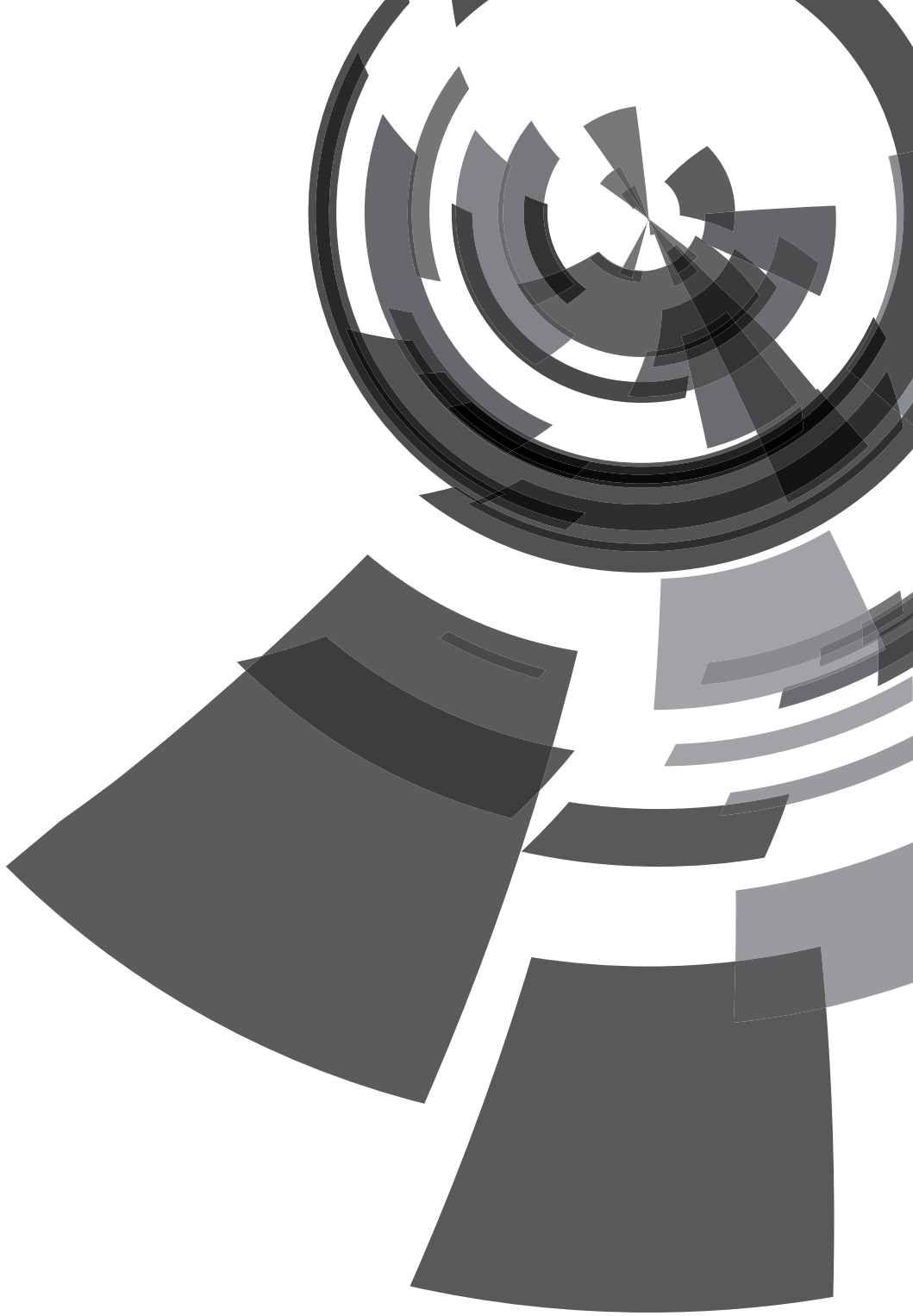


Are Nutrition Labels Always Correct?

Authors: Sahrish Khan & S. Alisha Sollis
Mentor: Afshin Ghoreishi

Abstract: Consumers generally believe labels on items they buy. In particular, they increasingly rely on Nutrition Facts labels to make informed food buying decisions. Although no label is expected to be one hundred percent precise, they should not be incorrect. Our suspicion was raised about HyTop medium olives when by a chance visual inspection of a six ounce can it was clear that it could not possibly hold the number olives stated on its Nutrition Facts label. For our statistical study, we tested six ounce cans of HyTop medium and large black olives, which are the only two olive sizes the company markets, for both the number of olives and their weights stated on their Nutrition Facts labels. Using a t-test with large random samples, we found that the stated number of olives and their weights are incorrect by a statistically significant amount. As suspected, the label for the medium olives is an overstatement of both the number and weight of olive. But, surprisingly, the label for the large olives is an understatement of the number of olives while it is an overstatement of the weight of olives. We can explain these inconsistencies by a possible typographical error on the label of cans of medium olives and not using large enough olives in the cans of large olives. We are sending a copy of this study to the HyTop company with suggestions for correcting these errors. In order to do a future study using small samples, we also tested these cans of olives for normality of the distribution of both number and weight of olives. Using normal probability plots, we determined that indeed the number of olives and weight of olives in both sizes are normally distributed.

Introduction

This study started with a comment of a friend of our advisor that it is not physically possible for the six ounce can of HyTop medium black olives, which he was about to open, to hold the number of olives stated on its label. We decided to test both HyTop brand medium and large size black olive six ounce cans for the weight and number of olives. Also, we show that both the number of olives and their weight are normally distributed. This allows us to repeat this study in future using small samples.

Methods

Sampling

To test our hypothesis we used random sampling. We bought a total of thirty, six ounce cans of each of medium and large size black olives from two locations. We counted number of olives in each can and weighed the olives (dry weight). See Appendix.

Hypothesis Testing

Since we did not have the population standard deviation and also didn't know if the population was normally distributed, we used one sided t tests to test our hypotheses.

H_o:$\mu=\mu_0$ versus H_a:$\mu<\mu_0$ or H_a:$\mu>\mu_0$, where μ_0 is the average number of olives per can or is the average dry weight of olives. The test statistic is

$$t = \frac{\bar{x}-\mu_0}{s/\sqrt{n}}$$

where $\bar{x}$ denotes the sample mean, $s = \sqrt{\frac{\sum_{i=1}^{n}(x_i-\bar{x})^2}{n-1}}$

is the sample standard deviation and n=30 denotes the number of cans. In each case, we state our conclusion along with the t-value and the corresponding p-value. (Mendenhall, Beaver & Beaver p. 273 -280).

Assessing Normality

To assess whether the number of olives per can or the weight of olives per can are normally distributed we draw a normal probability plot for each of the four cases. This is done by first putting the data in ascending order and using the position of each data value i to calculate the expected proportion of data less than or equal to each data value, $f_i = \frac{i-0.375}{n+0.25}$,

with n=30, then from the standard normal table the z-scores corresponding to areas to their left equal to the f_i values are obtained. The normal probability plot is the plot of the data values versus their corresponding z-scores. If the data is normally distributed, this plot will be approximately normal. To better judge the linearity, we will also show both the expected straight line the data should fall on and its 95% confidence interval. (Sullivan Section 7.4).

Hypothesis Tests Results

I. Our first test is to determine whether the number of olives in the six ounce HyTop cans of medium size black olives is overstated or not. The can label claims each serving is 5 olives and the can contains 20 servings. See the corresponding nutrition label in the appendix on the next page.

H_o:μ=100 versus H_a:μ<100

The average number of olives was calculated to be x=58 with the standard deviation of s=2.79. The t-value is t=- 82.45. Therefore, we reject the null hypothesis since the p-value, for the t distribution with n-1=29 degrees of freedom, is p=0.

II. Our second test is to determine whether the dry weight of olives in the six ounce HyTop cans of medium size black olives is overstated or not. The can label claims each serving is 15 grams and the can contains 20 servings. See the corresponding nutrition label in the appendix below.

H_o:μ=300 versus H_a:μ<300

The average dry weight of olive cans was calculated to be x =167.81 with the standard deviation of s=7.94. The t-value is t=-91.19. Therefore, we reject the null hypothesis since the p-value, for the t distribution with n-1=29 degrees of freedom, is p=0.

III. Our third test is to determine whether the number of olives in the six ounce HyTop cans of large size black olives is understated or not. The can label claims each serving is 4 olives and the can contains 11 servings. See the corresponding nutrition label below.

H_o:μ=44 versus H_a:μ>44

The average number of olives was calculated to be x=47.67 with the standard deviation of s=3.94. The t value is t=5.10. Therefore, we reject the null hypothesis since the p-value, for the t distribution with n-1=29 degrees of freedom, is p≈0.

IV. Our last test is to determine whether the dry weight of olives in the six ounce HyTop cans of large size black olives is overstated or not. The can label claims each serving is 15 grams and the can contains 11 servings. See the corresponding nutrition label below.

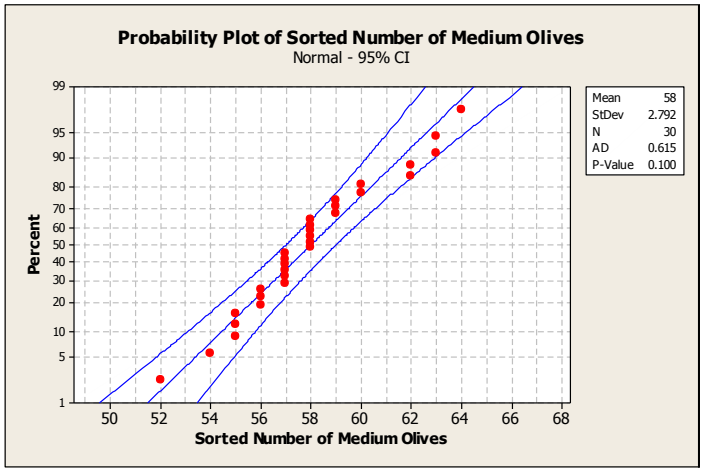
H_o:μ=165 versus H_a:μ<165

The average dry weight of olive cans was calculated to be x=155.95 with the standard deviation of s=7.85. The t-value is t=-6.31. Therefore, we reject the null hypothesis since the p-value, for the t distribution with n-1=29 degrees of freedom, p≈0

Normal Probability Plots

The normal probability plots below are done using MiniTab. For the f_i values and z-scores of the weights, see the tables in the appendix. All four normal probability plots are approximately normal and within 95% confidence interval. Therefore, the number and weight of olives in the six ounce cans of HyTop medium and large black olives are normally distributed.

I.



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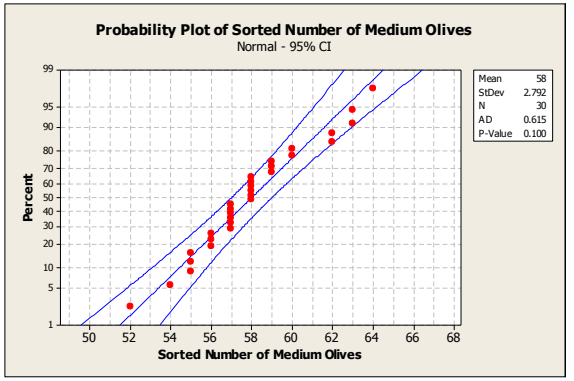

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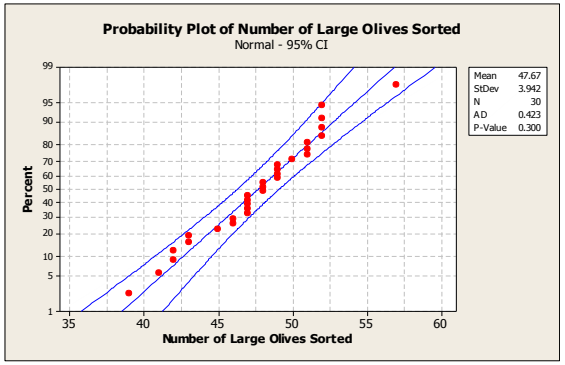

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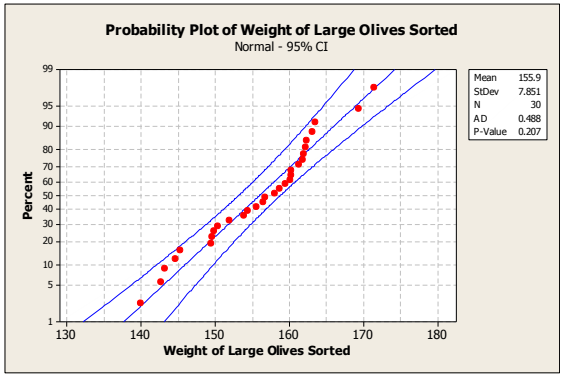

Conclusions

Since in all four tests, the null hypothesis is rejected with p≈0, in three cases the label information is a significant understatement while in the other case it is a significant overstatement. We hope that the error in part of the medium olive cans is just a typographical error. However, our only explanation for the error in the large olive cans is that the olives are not large enough. We have sent a copy of this study to the HyTop company with the suggestion of labeling the cans of medium size olives with 11 servings per can and serving size of 5 olives while using larger olives in the cans of large size olives. If and when corrections is made to the labels of this products, we can re-test them using small sample hypothesis testing.

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WinCo Stores: 205 WEST 12th St., Ogden, UT 84404 and 6060 South 3500 West, Roy, Utah 84067.

Appendix

Table 1 – HyTop Medium Black Olives, Six Ounce Cans

Product Number	Number of Olives	Weight	i	f_i
110HP/20AA0616	53	204.97	1	0.020661
110HP/41F1800	54	208.84	2	0.053719
110HP/41GG0231	55	212.7	3	0.086777
110HP/27BB0803	55	212.7	4	0.119835
110HP/41F1755	56	216.57	5	0.152893
110HP/41GG0111	56	216.57	6	0.18595
110HP/27BB0851	56	216.57	7	0.219008
110HP/41GG0232	56	216.57	8	0.252066
110HP/41F1841	57	220.44	9	0.285124
110HP/41F1758	57	220.44	10	0.318182
110HP/41GG0110	57	220.44	11	0.35124
110HP/20E1711	58	224.31	12	0.384298
110HP/41F1756	58	224.31	13	0.417355
110HP/20E1639	58	224.31	14	0.450413
110HP/41F1844	58	224.31	15	0.483471
110HP/41F1757	58	224.31	16	0.516529
110HP/41F1842	59	228.17	17	0.549587
110HP/20HH2358	59	228.17	18	0.582645
110HP/110AA0648	59	228.17	19	0.615702
110HP/41F1813	60	232.04	20	0.64876
110HP/27B0851	60	232.04	21	0.681818
110HP/20E1520	60	232.04	22	0.714876
110HP/41GG0110	60	232.04	23	0.747934
110HP/20E1638	61	235.91	24	0.780992
110HP/91G0109	61	235.91	25	0.81405

Table 1. Continued

100HPF/317II0003	61	235.91	26	0.847107
100HPF/317II2324	61	235.91	27	0.880165
110HP/20E1521	62	239.78	28	0.913223
110HP/20E1640	62	239.78	29	0.946281
110HP/110AA0647	62	239.78	30	0.979339

Table 2 – HyTop Large Black Olives, Six Ounce Cans

Product Number	Number of Olives	Weight	i	f_i
110LP/34F1619	45	171.549	1	0.02066116
410LP/73GG2119	47	179.174	2	0.05371901
410LP/73GG2120	47	179.174	3	0.08677686
110LP/89F1721	47	179.174	4	0.11983471
110LP/41C1243	47	179.174	5	0.15289256
110LP/41C1235	47	179.174	6	0.18595041
410LP/73GGG2121	48	182.986	7	0.21900826
110LP/34F1620	48	182.986	8	0.25206612
110LP/41C1245	48	182.986	9	0.28512397
110LP/41C1242	49	186.798	10	0.31818182
410LP/73GG2128	49	186.798	11	0.35123967
410LP/74GG2033	49	186.798	12	0.38429752
110LP/89F1722	50	190.611	13	0.41735537
110LP/89F1720	50	190.611	14	0.45041322
110LP/89F1621	50	190.611	15	0.48347107
110LP/48AA0658	50	190.611	16	0.51652893
110LP/41C1209	50	190.611	17	0.54958678
110LP/41C1241	50	190.611	18	0.58264463
110LP/41C1234	50	190.611	19	0.61570248
110LP/96HH2141	51	194.423	20	0.64876033
110LP/94B0810	51	194.423	21	0.68181818
110LP/96HH2137	51	194.423	22	0.71487603
110LP/89E1649	52	198.235	23	0.74793388
110LP/89E1647	52	198.235	24	0.78099174
110LP/89E1648	52	198.235	25	0.81404959

Table 2. Continued

110LP/96HH2142	53	202.047	26	0.84710744
110LP/96HH2133	53	202.047	27	0.88016529
110LP/96HH2136	54	205.859	28	0.91322314
110LP/41GG0109	56	213.484	29	0.94628099
110LP/41C1246	57	217.296	30	0.97933884

Photo of a can of HyTop medium black olives

Photo of a can of HyTop large black olives

Analysis of First and Second Drops: Is the First Drop of Blood Truly Necessary with the Coaguchek XS Analyzer?

Author: Mandy Griego

Mentor: Kara Hansen-Suchy

Abstract: This study addressed if a significant difference exists between the first and second drops of capillary blood when using the Roche CoaguChek XS analyzer. This handheld analyzer is used by healthcare professionals to measure the time it takes patients on anticoagulation therapy to form a blood clot. Anticoagulation therapy can prevent life-threatening blood clots due to previous health problems, including heart attack, stroke, or heart valve surgery. Over-medicated patients risk fatal internal bleeding, while under-medicating can result in blood clot formation within the body, making monitoring critical for maintaining proper medication dosage. The CoaguChek XS instructions emphasize using the first drop of capillary blood; however, no rationale is provided. The first drop of blood can be difficult to collect, so contrary to procedure, the second drop is often analyzed. Participants in the study were sixty-six volunteers not on anticoagulants. They donated capillary blood, and clot times were analyzed for the first and second drops of blood. No significant differences between first and second drops were found, leading to the conclusion that the use of a subsequent drop is acceptable. These findings can be used to educate users of the CoaguChek XS and assure the quality of the reported results.

Introduction

There are many individuals on blood thinners to prevent life-threatening internal blood clots. These anticoagulation medications are also known as Warfarin or Coumadin therapy. Patients who are taking anticoagulation medication are typically those who have experienced heart attacks, strokes, congenital heart defects, abnormal heart rhythms, or heart valve surgery (Gallus, Alex S., et al., 2000). The dosage of medication these individuals are on corresponds to routinely monitored prothrombin (protime) or clotting times. Protimes are converted into standardized values using the International Normalized Ratio (INR). These INR values are reported to physicians and represent accurately measured clotting times no matter the laboratory method or instrument used. These anticoagulation therapies are essential in preventing and treating venous or arterial thrombosis and embolism. The potential effects of such drugs include major bleeding due to overmedicating the patient and can be very dangerous. Each patient has a different dosage which is necessary to prevent these risks, and their prescribed amount is constantly being adjusted. A balance is maintained to keep the patient medicated enough to prevent blood clots, but not to the point of causing them to bleed internally (Gallus, Alex S., et al., 2000).

Most patients undergo weekly venipuncture blood draws for this monitoring. For some, collection by this method can be consistently difficult due to small or deep veins. Hospitals and clinics can elect to use a handheld protime analyzer called the CoaguChek XS System. Extensive studies have been done to validate the CoaguChek XS systems as being accurate in comparison to other methods used in large hospital laboratories (Plesch, W., et al., 2008). The CoaguChek XS system is located at the site of patient care and gives results to be reported to the doctor almost immediately using a finger-stick method instead of venipuncture. A small test strip that contains chemical reagents specific for stimulating blood to clot is first inserted into the analyzer. The patient's finger is then pricked, and a drop of capillary whole blood is placed onto the test strip and read by the analyzer (Longair, I., et al., 2006).

The instruction manual of the CoaguChek XS mentions not exceeding fifteen seconds before applying blood to the test strip, implying the drop of blood used should be fresh. Additionally, the necessity of using the first drop of a patient's capillary blood is strongly emphasized (Longair, I., et al., 2006). The first drop of blood sometimes is not used due to the difficulty of collection onto the test strip. The goal of this project will be to examine if there is a clinically significant difference between the first and second drop of capillary blood when using this analyzer.

If utilizing second drops is acceptable, there would not be a need for a second capillary stick nor the use of an additional test strip. Because the dosage of medication is dependent on the results of the monitored protimes, the accuracy of such analyzers as the CoaguChek XS is critical. If there is no clinically significant difference between the drops, the use of the second drop would not compromise patient care.

Materials and Methods

Sixty-six healthy volunteers ranging from ages eighteen to sixty-eight were recruited at Weber State University campus by advertising compensation in exchange for participation. Capillary blood was collected from participants by the finger stick method using CoaguChek brand lancets specific for the CoaguChek XS analyzer. Each volunteer's first drop of blood was taken from one finger and collected onto a CoaguChek XS test strip that had been inserted into the CoaguChek XS analyzer and the subsequent INR and

prothrombin time were recorded. From an additional finger, the first drop of blood was discarded and the second drop of blood was collected onto the test strip and recorded.

On the occasion that collection onto the test strip did not work for a participant's second drop, the patient's blood flow was kept fresh by constant finger massaging and wiping until the analyzer was ready for another reading. If collection did not work for a participant's first drop time, a new finger was pricked. This process was repeated for all sixty-six subjects. Once results were compiled, all data were statistically analyzed for significant differences.

All subjects were assigned a random accession number corresponding to a master copy with the subjects' names, ages, prothrombin times, and INRs. This master copy was given to our principal investigator and held under lock and key in their private office for the duration of our study.

Results

Table 1. The results for each subject were analyzed using four paired t-tests and one ANOVA.

Analysis test ran:	P-value	Interpretation
T-test 1: Protimes of 1st and 2nd or subsequent drops for all 66 subjects	0.316	**No significant difference**
T-test 2: INRs of 1st and 2nd or subsequent drops for all 66 subjects	0.304	**No significant difference**
T-test 3: Protimes of 1st and only 2nd drops (excludes 8 results obtained after failure to capture second drop)	0.153	**No significant difference**
T-test 4: INRs of 1st and only 2nd drops (excludes 8 results obtained after failure to capture second drop)	0.310	**No significant difference**
ANOVA: Protimes of 1st drop, 2nd drop, and additional/subsequent drops.	0.803	**No significant difference**

(p=0.01)

Discussion

These results indicate that there is no significant difference between a first, a second, or even a subsequent drop of blood on the analyzer. Subsequent drops occurred when a drop of blood was discarded because of a misreading of the strip or load error. The drop of blood from a volunteer was kept fresh by lightly massaging the finger and wiping the drops of blood. In the meantime, a new test strip was inserted and the analyzer took about thirty seconds to one minute to reset itself before it was ready to analyze another drop. As soon as the analyzer indicated it was ready to perform the test, a final drop of blood was wiped and the next full drop was applied to the test strip. These subsequent drops were included in Table 1 with the first and second t-tests ran, but were excluded in the final two t-tests. They were included as a separate subset in the ANOVA.

The original intention of this study was to include a group of individuals on anticoagulation medication that would be approximately thirty percent of the total sample size. This would allow for the possibility of high protime and INR values for both drops of blood to analyze and determine if the values still show no significant difference. The timeframe of this study did not allow for the application process to make it through the Institutional Review Board for permission to gain results from medicated volunteers at a local retirement center.

Results from sixty-six volunteers were used, however the initial sample size desired was 100 individuals. At the beginning of testing, funding was available for 192 test strips, meaning ninety-six individuals' first and second drops of blood could be analyzed. Difficulties that were encountered in running the CoaguChek XS included the capillary action of the blood being pulled under the test strip instead of on top, the blood drop not being an adequate size to induce a reaction with the test strip, and the strip not drawing the drop of blood into it. Because of these difficulties throughout the study, about sixty test strips were discarded without giving a reportable value, decreasing our sample size.

The results of this study indicate that on the occasion that an operator of the CoaguChek XS accidentally wipes or inadequately captures the first drop of blood, the second drop of blood, and even previously mentioned subsequent drops of blood, the machine will still give comparable values. This will eliminate the need for an additional finger stick, a very uncomfortable process for many patients. The additional cost of extra supplies that a facility

may use, including the specific CoaguChek XS lancets, would also be spared. Future studies could possibly include an investigation of the following variables 1) extended amounts of time in relation to drop values, 2) an increased sample size, 3) an extension of value ranges (e.g., a significant

sample size of volunteers on anticoagulation therapy) and 4) an exact estimate of supply costs saved due to this information.

Conclusion

From these results, a conclusion may be reached that the difference between a patient's first and second drops of blood on the CoaguChek XS analyzer is statistically insignificant. There is essentially no difference between drops, therefore the second drop protime and INR values are acceptable to report.

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Pertussis Immunity in a Sample Population of Pregnant Women

Author: Dana Heiner & Rachel Bown
Mentor: Kara Hansen-Suchy

Abstract: Recent outbreaks of pertussis, more commonly known as whooping cough, and a steady increase of nationwide reported cases, have brought attention to a once common childhood disease. Prior studies have demonstrated that mothers with immunity to pertussis will transfer a comparable amount of immunity to their newborns, giving them some protection until immunizations can begin. In consideration of this, it is important to determine if pregnant women have detectable levels of immunity to Bordetella pertussis. Blood samples were taken from ninety-four pregnant women age twenty-five and older to measure their levels of IgG to the pertussis antigen. Participants were also given a short survey regarding their pertussis immunization history. The testing was performed using an ELISA method specific for the pertussis IgG antibody. Only forty-five percent of the participants tested positive for the antibody, which was much lower than expected. In addition, a high percentage of the participants could not recall when they had last received a pertussis vaccination. These results suggest a need for public awareness and education about pertussis and the options for vaccination. This is crucial in preventing the spread of pertussis, not only for the general public, but particularly for mothers and their newborn children.

Introduction

Pertussis, more commonly known as whooping cough, is caused by *Bordetella pertussis*. This gram-negative fastidious bacterium is responsible for one of the most contagious childhood diseases. The pertussis organism produces several toxins. These toxins increase mucus production and damage the ciliated cells of the pharynx. They also cause constriction and hemorrhage of vessels and inhibit movement of the white blood cells. The damage to the cilia prevents effective clearing of mucus from the lungs. The body reacts with a severe, intense cough with 40-50 coughing spells each day. This cough can linger for as long as three months. Adults with pertussis can experience mild symptoms and may not even be aware that they are infected. More severe cases of pertussis have symptoms that include malnutrition, apnea, pneumonia, encephalitis and even death (Edwards, 2003). Children under the age of one year are at highest risk for experiencing the most devastating outcomes of this disease (Helmy, 1992).

After DTP vaccine (to prevent diphtheria, tetanus, and pertussis) was introduced in the 1940's, the incidence of pertussis dramatically decreased. The rate decreased from over 250,000 cases per year to 1,010 cases reported in 1976. In 1991, the DTaP vaccine was licensed for use. 2005, two new pertussis vaccines were licensed for use in adolescents and adults; both abbreviated Tdap. The original DTP was made using the whole pertussis organism, but the new DTaP and Tdap were made using small antigenic subunits of the pertussis organism (Pertussis, 2008). These newer vaccines resulted in fewer side effects. Despite preventive measures, the incidence of pertussis has increased nationwide over the last several years (Vaccines and Preventable Diseases, 2010). Some reasons for this increase are: parents refuse to vaccinate their children because of the fear of side effects, (Bauman, 2007) and acquired immunity from the pertussis vaccine only lasts about five to ten years (Murphy, 2008).

In addition to this, the DTaP vaccine is only 59-89 percent effective in preventing pertussis; so pertussis can occur in people who have received the vaccine, but the disease is less severe and has fewer complications (Pertussis, 2008).

In 2010, many states reported increases in pertussis cases with an epidemic reported in California that resulted in 10 infant deaths (Vaccines and Preventable Diseases, 2010). In Utah, health officials are concerned that an outbreak is due in the Salt Lake Valley. Dr. Dagmar Vitek with the Salt Lake Valley Health Department stated, "We usually see increased pertussis numbers every three to five years, we are probably due for an outbreak about now (Leonard, 2011)." The last outbreak in Utah was in 2006, when the Salt Lake Valley experienced a 400 percent increase in reported cases (Leonard, 2011).

Due to the susceptibility of young children to this disease, it is very important for adults to be immunized, and more significantly for pregnant mothers to be immunized, either before pregnancy or immediately postpartum. The significance of this is two-fold. First, mothers are the primary caregivers for their children. If a mother is not sufficiently immunized, she can become infected and pass the disease agent to her infant. Second, if a pregnant mother has immunity, some immunity can be passively transferred transplacentally from mother to child while that child is yet unborn (Edwards, 2003). This immunity is only temporary, but gives some protection to the infant until they are old enough to start their vaccinations. The recommended age for the first DTaP dose is two months old, and the mother's immunity that is passed to her infant can last up to four months (Edwards, 2003).

Materials and Methods

The subjects were tested for the IgG antibody for *Bordetella pertussis* to determine immunity levels. The target population was pregnant women who were patients at the Circle of Life Women's Center in Ogden, Utah. Patients considered high risk were excluded from the study. This included women with pre-eclampsia, chronic illness, heart disease, etc. All of the participants were already scheduled for routine blood work.

The participants were given a survey with some general questions about their immunization history and a consent form to sign that was approved by the Weber State University Institutional Review Board. Then they were asked to donate an extra tube of blood, along with the blood work already ordered by their doctor. This was drawn into 3.5 mL serum separator tube. The tubes were allowed to clot for ten minutes and then centrifuged for ten minutes. The serum was poured into transport tubes and kept frozen until sent for testing.

The testing of the sera was performed at ARUP Laboratories using an Enzyme Linked Immunosorbent Assay method. The method uses a 96-well microplate. Each sample was tested in duplicate which required two 100uL serum samples from each participant. The samples were each pipetted into two separate wells of a microplate. The microplates were incubated and washed three times. Conjugate was added to the sample wells, then incubated and washed three times. Substrate was added to the wells and incubated. Stop solution was added to the wells and the microplates were mixed by agitation. The results of each well were read and interpreted by a microplate reader using a 450 nm wavelength. The results are semi-quantitative and given in one of three ranges. 0.0 - 0.9 U/mL is considered a negative result. 1.0 – 2.4 U/mL is considered equivocal and 2.5 U/mL or greater is considered a positive result.

Samples that were hemolyzed, showed indications of bacterial contamination, or samples less than 1mL in volume were rejected. Two samples were rejected because they were short samples. One was rejected because of hemolysis and two samples were rejected because the participants did not sign their consent forms. A total of ninety-four samples were sent for testing.

Results

The results were given in three semi-quantitative ranges measured in U/mL that indicated a positive, equivocal or negative result. The positive range includes a result of 2.5 U/mL or greater. The equivocal range is 1.0 – 2.4 U/mL, and the negative ranges is 0.0 - 0.9 U/mL. Of the 94 participants tested, 45% tested in the positive range, 22% tested in the equivocal range and 33% tested in the negative range for pertussis IgG antibodies. Refer to Figure 1.

The age range of the participants was between 25 and 37, with the average age being 29. There was no correlation between the ages of the participants and their IgG pertussis levels. The participants were also given a short survey asking basic questions about their pertussis immunization history. The survey indicated that 65 of the 94 participants had been vaccinated for pertussis. Of those participants, only 30 tested positive for a level of IgG antibodies indicating protective immunity. The survey also showed that 13 of the 94 participants claimed that they had never been vaccinated for pertussis. Of those participants, 7 tested positive for a protective level of IgG antibodies. The survey also indicated that 16 of the participants did not know whether they had ever been vaccinated for pertussis. Of those participants, 5 tested positive for a protective level of IgG antibodies.

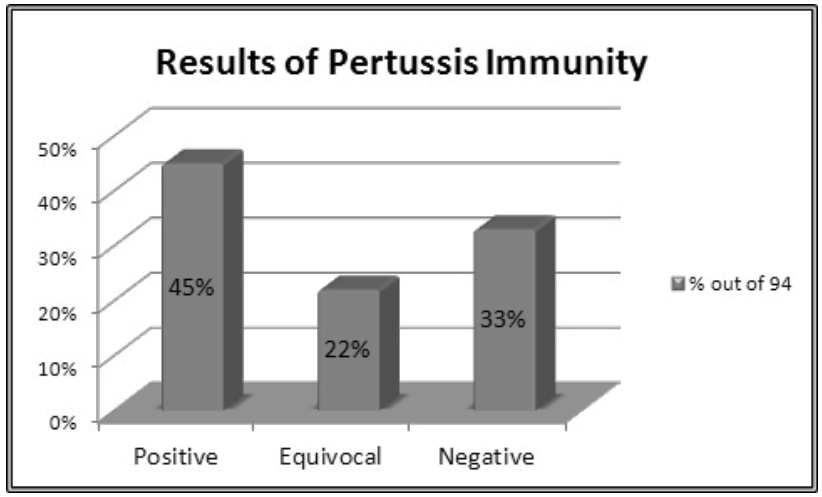

Figure 1. Results of Bordetella pertussis IgG antibody test.

Discussion

This project evaluated whether pregnant mothers along the Wasatch Front have protective levels of IgG antibodies immunity to *Bordetella pertussis*. As seen from the results, a significant percent do not have adequate immunity. This percentage was much higher than expected and considering the repercussions of not having immunity, these numbers are quite disturbing.

Another unexpected aspect of this research was the lack of awareness about pertussis that was shown by a majority of the participants. When speaking with the participants, many did not know what pertussis was or. If they had ever been vaccinated for pertussis. A number of the participants that did not

the corpus luteum to continue secreting progesterone in the ovary. believe they had ever been vaccinated or awareness that they ever had the disease, yet they tested positive for the antibody. (These IgG antibodies could have been present due to infection with pertussis or due to vaccination.) In general, this clearly indicates a lack of awareness about their personal history of pertussis vaccination or of a history of pertussis disease in their past..

The method used for testing in this research, is one that can be used to test for active disease. It is unknown if the antibodies that give a positive reaction to this test are acquired from vaccination or from exposure to the disease. Other methods test for IgM or IgA antibodies, toxins to the disease, or even test for the pertussis organism provide a way to clarify which situation it is.

For the purposes of this research, the IgG antibody test was the most logical, considering that it is present in long term immunity and the purpose of this research was to test for pertussis immunity.

Conclusions

Based on the results of this research, women in Northern Utah have a low percentage of immunity to pertussis. This puts infants and young children in their care at risk for infection with a possibility of devastating outcomes. It is also evident that there is a need for further public education on this disease and the importance of adult immunization. Physicians and health providers have a duty to educate their patients about pertussis and the importance of adults keeping their immunizations current.

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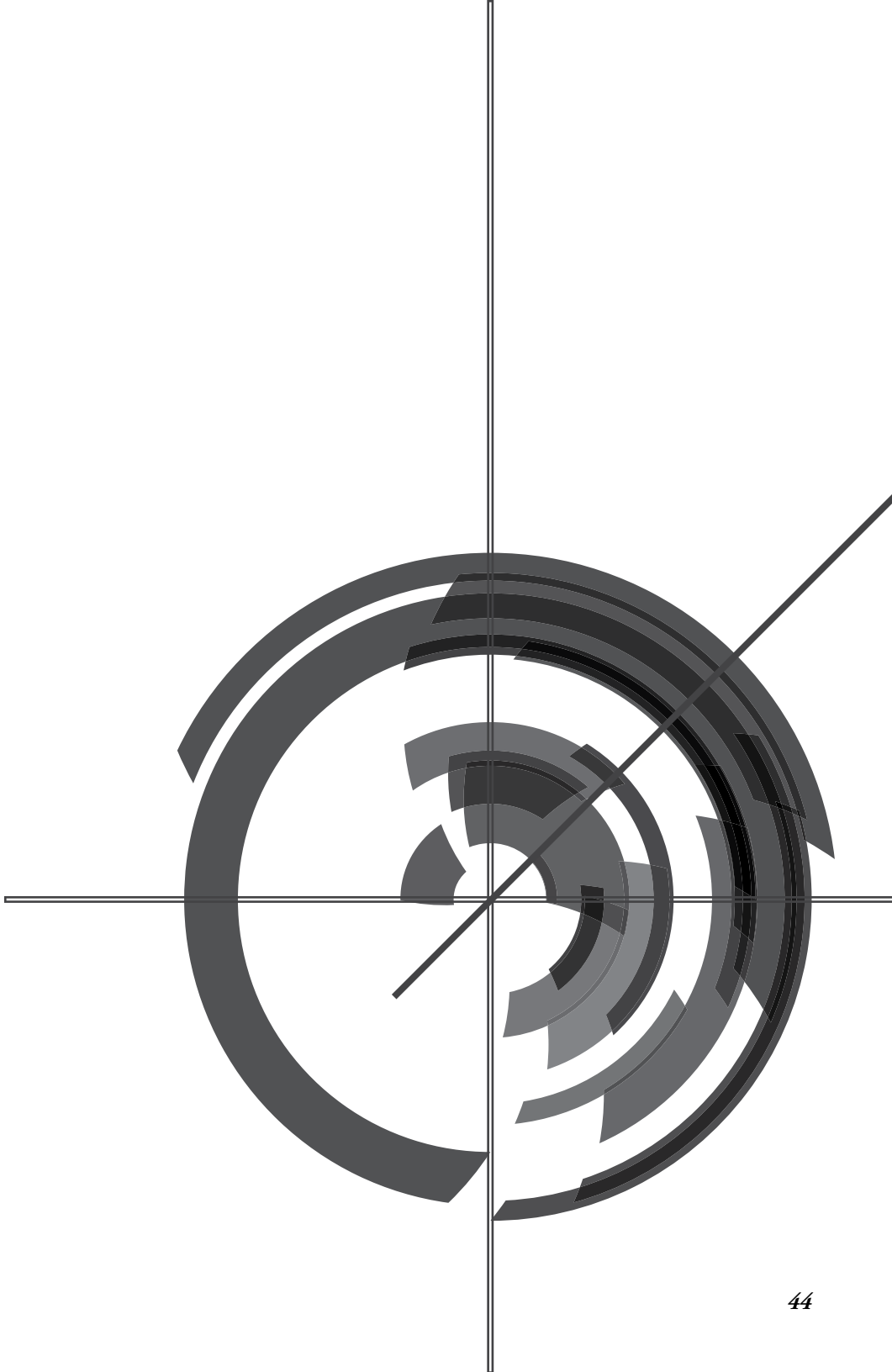

The Effects of hCG Diet Injections on Pregnancy Tests

Authors: Cory Woolsey & Jaren Goff
Mentor: Gary Nielsen

Abstract: The purpose of this study was to determine if hCG diet injections cause non-pregnant women to obtain unreliable pregnancy test results. Common clinical and home pregnancy tests detect levels of the hCG hormone in the blood or urine. The hCG pregnancy hormone is the same hormone used in the hCG diet. This study consisted of 15 non-pregnant and non-menopausal women who were selected from an hCG diet clinic in Springville, Utah. Participants were selected based upon answers given in a preliminary questionnaire. All participants were tested before or after their course of exogenous-hCG injections to establish a control. Experimental data were obtained by performing pregnancy tests on the participants' blood and urine while they were taking their course of exogenous-hCG injections. Experimental data showed that of the 15 women that participated in this study all tested positive on the serum hCG test and 14 of the women tested positive on the urine hCG test. These results indicated that exogenous-hCG injections can cause non-pregnant women to obtain unreliable pregnancy test results. This information would be useful for physicians and those who participate in injectable hCG diets.

Introduction

The purpose of this study was to determine if hCG diet injections cause non-pregnant women to obtain unreliable pregnancy test results. The information that supports this study is divided into four subsections within the introduction of this paper, in the following order: *Biological-hCG, The hCG Diet, The Pregnancy Test,* and *The hCG Connection*.

Biological-hCG

Human chorionic gonadotropin (hCG) is a hormone that is naturally produced by the pituitary gland of all humans. It is also produced by developing embryo blastocysts, and the placenta in pregnant women. The hCG from these sources can be found and tested for in blood and in urine. Excluding the timeframe of menopause, the pituitary gland secretes very small amounts of hCG that should never be detected during a pregnancy test. During menopause the pituitary gland can secrete enough hCG to cause a pregnancy test to turn positive. Developing embryo blastocysts produce far more hCG than the pituitary gland. Embryonic-hCG is produced to cause

Continued progesterone secretion is vital to prevent regular menstruation which allows the embryo to grow in the womb. Tumors and cancers such as hydatidiform moles, choriocarcinoma, and testicular germ cell tumors may also secrete large amounts of hCG (Yoshimoto, Wolfsen, & Odell, 1979).

Numerous studies show that contraceptives are also known to cause an increase in hCG production, but generally do not elevate the hCG level high enough to be of concern for the purpose of this study. The source of hCG during contraceptive use is believed to be from the pituitary gland. An example of this would be the study made on the effect of an intrauterine contraceptive on hCG levels. The serum hCG levels obtained in the study were all less than three milli-International Units per milliliter (3 mIU/mL) (Shalit, Jaschevatzky, Kampf, Leiserowitz, & Grünstein, 1980).

Three molecular forms of hCG exist. 1) Regular hCG; 2) glycosylated-hCG, hCG covered with greater concentrations of carbohydrates than does regular hCG; 3) free-hCG, hCG that is free of carbohydrates. Embryo blastocysts; hCG producing tumors and cancers; and pituitary glands all vary in how they attach carbohydrates to hCG. The altered attachments of carbohydrates can be used to tell the difference (differentiate) between the origins of the hCG molecules (Yoshimoto, Wolfsen, & Odell, 1979). However, common clinical and home pregnancy tests cannot differentiate between the regular hCG, glycosylated-hCG, and free-hCG. Therefore, for the purpose of this study, all three isoforms created by the body will be referred to as "biological-hCG."

The hCG Diet

The hCG diet was originally created by Dr. Simeons in the 1950's. His method of the diet consists of a restriction of calorie intake to 500 kilocalories (Calorie or Cal) per day, while injecting hCG into the thigh or stomach fat with a small needle. The injectable form of hCG used in the hCG-diet will be referred to as "exogenous-hCG" throughout this paper; exogenous meaning "from without." An oral administration of exogenous-hCG exists for the hCG diet, but for the purpose of this study only the use of injectable hCG will be examined.

When an adult performs a 500 Cal diet without the additional intake of exogenous-hCG, hunger and breakdown of muscle tissue are naturally experienced before fat tissue is broken down for energy. According to Dr. Simeons' theory, the addition of exogenous-hCG to a 500 Cal diet allows an adult to experience an increased release of stored triglycerides from fat tissue into the blood. Increased weight loss results from the triglycerides being released from fat tissues and being utilized for energy in the body (Simeons, 1954). Exogenous-hCG is made by collecting the urine of pregnant women, then isolating and purifying biological-hCG to a level safe enough to be

injected into a human (Novarel, 2011). This means that exogenous-hCG is the same hCG hormone found in the urine of pregnant women. Biological and exogenous-hCG are identical in structure and source.

The Pregnancy Test

The hCG hormone is composed of two protein subunits known as alpha (α) and beta (β). The β-hCG-subunit is unique in design and is sought after as a binding site for testing purposes to create a more sensitive pregnancy test (Cole, 2009). The most common forms of clinical and home pregnancy tests are based upon an immunological reaction between the anti-β-hCG antibody, its antigen β-hCG, and colloidal gold-labeled hCG reagent molecules.

When biological-β-hCG is present in concentrations above the measurable threshold, a positive indicator becomes visible on the pregnancy test. No color change occurs on the test if there is an insufficient concentration of hormone present; hence the test is interpreted as being negative (Sure-Vue®, 2008). After searching through research articles the minimum detection level values for pregnancy tests were found to range between 5 mIU/mL and 50 mIU/mL.

Common clinical and home pregnancy tests are qualitative tests. They only report whether or not there is a minimum amount of hCG hormone present in the woman to turn the test positive. When the test turns positive this may indicate that the woman is pregnant. This must be confirmed with a physician and a follow up pregnancy test. The tests do not differentiate between biological-hCG and exogenous-hCG. If a test says that a woman is pregnant, and she is *not* pregnant, then the test is considered to be "false-positive."

The hCG Connection

If the β-hCG pregnancy test assays for the β-subunit of biological-hCG, then it is expected that the β-subunit of exogenous-hCG will also react with the anti-β-hCG antibodies of the pregnancy test. This means that if a woman takes exogenous-hCG injections, and her body's concentration of exogenous-hCG is high enough, a common β-hCG pregnancy test of her urine or serum should result false-positive. This is exactly what happened with Noci et al. (1987), where pregnancy testing was performed on women injected with exogenous-hCG for ovulation inducement. Noci et al. injected 5,000,000 mIU of exogenous-hCG on days 3, 5, and 7 after ovulation and obtained false-positive pregnancy test results up to day 12 using pregnancy tests of 50 mIU/mL sensitivity. Currently, there is no data available that proves that an exogenous-hCG injection regimen used for the hCG diet could cause a false-positive pregnancy test. If false-positive pregnancy tests

show up during testing for this study, then the information would be useful for physicians and those who participate in the hCG diet. As seen from the results and conclusion of this paper, false-positive pregnancy tests do show up during the course of an injectable hCG diet.

Materials and Methods

Approval was obtained from the Weber State University Internal Review Board for Human Subjects prior to experimentation.

Fifteen women participated in this study after they agreed to, and signed, an informed consent document. The women reported themselves as being non-menopausal and non-pregnant, and as never having had an hCG producing cancer. The women were allowed to use contraceptives, and were in general good health (they had a pre-diet health examination to determine if they could participate), and were allowed to participate in sexual intercourse. Questionnaires were used before sample collections to determine whether or not the women's samples could be included in the study.

The women were divided into two groups in order to include as many women as possible into the study. Group A consisted of nine women who had baseline sample collections taken prior to starting their hCG diet. Group B consisted of six women who had baseline sample collections taken at least three days after ending their hCG diet. Both groups A and B had their experimental samples taken at least a week into their course of daily exogenous hCG injections.

The Calorie intake of these women was unimportant to this study. The women injected themselves each morning with 0.2 mL of Novarel solution, concentrated to 1,000,000 mIU/mL. This means that the women each self-administered a total amount of 200,000 mIU of Novarel per day. Novarel is an exogenous-hCG drug that is approved by the Department of Food and Drug Administration (FDA). An FDA approved drug was used so that the reported concentration of the exogenous-hCG drug would be more reliable than that of a non-FDA-approved product.

The baseline and experimental samples each consisted of one serum sample and one clean-catch urine sample that was collected around 6:00 PM (6-12 hours after their morning injection of exogenous hCG). The serum was collected using a Serum Separator Tube (SST) via standard phlebotomy and specimen-processing procedures. A sterile urine-cup was used for urine collection, and each woman was given instructions on how to perform a clean catch urine sample. No further preparation was made to the serum and urine samples.

Testing of the serum samples for the presence of hCG was done using a clinical pregnancy test that had a sensitivity of 25 mIU/mL. The urine

samples were tested for the presence of hCG using an over-the-counter dipstick pregnancy test that had a sensitivity of 13 mIU/mL (Butler 2001). These two pregnancy tests were used to simulate the common qualitative pregnancy tests that are used in the United States.

Women who tested positive for hCG during baseline testing were excluded from the study. Women, who tested negative for hCG during baseline testing, remained in the study, and their experimental pregnancy test results are reported in the next section.

Results

Fifteen women were evaluated in this study. All fifteen women returned false-positive serum pregnancy test results. Fourteen of the women returned false-positive urine pregnancy test results. One woman returned a negative urine pregnancy test result.

Table 1. Number of false-positive and true-negative experimental pregnancy tests results.

hCG Diet Participants' Experimental Pregnancy Test Results		
Test Results	**Serum (25 mIU/mL)**	**Urine (13mIU/mL)**
False-positive	15	14
Negative	0	1

Discussion

None of the baseline pregnancy tests displayed positive results. This means that at about 6:00 PM all women that participated within the study naturally did not have elevated biological-hCG levels sufficient to elicit a false-positive reaction. When collection was taken at about 6:00 PM during the women's course of exogenous-hCG injections, all serum samples tested positive for pregnancy. Fourteen of the 15 urine samples returned positive for pregnancy. This means that at the same time of day something caused these women to obtain positive pregnancy test results. The cause is believed to be the daily morning injection of 200,000 mIU of exogenous-hCG.

All hormones have metabolism half-lives. Since hCG is a hormone it is degraded over time within a participant's body over the course of a day, which means hCG concentrations should be highest right after exogenous-hCG injection and lowest right before the next morning hCG injection. There is a possibility that if the participants were tested for pregnancy prior to their next morning hCG injection that the pregnancy tests would have returned negative, but this was not tested. The false-positive pregnancy test results obtained in this study do indicate that the daily injectable hCG diet regimen of 200,000 mIU creates an environment that elicits unreliable pregnancy test results.

The information gathered by this study would be useful for physicians and those who participate in injectable hCG diets. If a physician were to know that his or her patient was on an hCG injection based diet, and that this sort of diet influences the results of pregnancy tests, then the physician would know that the patient needs to stop the diet for at least three days to avoid obtaining inaccurate pregnancy test results. Participants of the injectable hCG diet, and their physicians are advised to not trust pregnancy test results until they have stopped the hCG injections for at least three days. This would help avoid the possibilities of an emotional rollercoaster caused by a positive pregnancy test when the test was really a false-positive.

Only injectable exogenous-hCG was examined in this experiment and because of this it can only be assumed that the use of oral exogenous-hCG would provide the same results. There is a possibility that oral administration of hCG provides results opposite of those found in this study.

Conclusion

Injecting 200,000 mIU of exogenous-hCG per day into a woman will cause her to have an unreliable, false-positive pregnancy test result.

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Biomedical Analyses of a Holistic Peanut Allergy Treatment: NAET

Athors: Brady Vincent, Dayne Bonzo & Dan McEntire
Mentor: Yasem Simonian

Abstract: Across the United States holistic medicine is frequently used as an alternative to or in combination with traditional medicine. One such treatment is for peanut allergies which involves a series of kinesiology and energy balancing methods. This rapidly growing treatment is called Nambudripad's Allergy Elimination Technique (NAET) was founded by Devi Nambudripad M.D., D.C., L.Ac, Ph.D, and is practiced primarily by licensed chiropractors. The differences seen in allergy treatment techniques applied by traditional versus holistic practitioners are based primarily on the definition of allergy. Although definitions and techniques may differ, the outcomes must be the same – that is, desensitization to the offending allergen. We hypothesize that applying NAET to individuals suffering from peanut allergies will be associated with a measurable decrease in the immunological response. If a participant becomes desensitized to the peanut allergen, it follows that there should be a measurable response. Participants with peanut allergies underwent an 18 week course of NAET and were tested for peanut specific IgE and IgG levels, as well as the inflammation marker tryptase. The blood was collected pre- and post-treatment and tested by validated methods at ARUP laboratories.

Introduction

The peanut allergy is one of the five most common food allergies. Many individuals seek and find relief from traditional medical allergists while others turn to alternative treatment methods. There are many options for holistic treatment methods and this study focuses on just one, Nambudripad's Allergy Elimination Technique (NAET). The purpose of this study is to evaluate a relationship between the practice of NAET and measurable immunology. We hypothesize that applying NAET to individuals suffering from peanut allergies will be associated with a measurable decrease in the immune response.

Dr. Devi S. Nambudripad developed NAET in November 1983. Since then, healthcare clinicians across the United States have incorporated her treatments into their practice. The treatment follows simple sets of techniques and protocols for allergens. Dependent upon the severity of the allergen, and when followed correctly, NAET claims that individual allergens may be eliminated in very few treatments. NAET protocol amends, however, that only one allergen can be treated at a time. If an individual is not seriously immunocompromised, each allergen can be alleviated by these techniques and treatments (Nambudripad D. , 2002).

Nambudripad's Allergy Elimination Technique is growing in popularity due to the claim that its practitioners can eliminate allergies and several other sensitivities. Nambudripad's treatment for any specific allergen is a composite of energy balancing techniques, clinical muscle response testing, acupressure, and diet schemes. NAET affirms the ability to desensitize individuals from allergens by non-invasive, pharmaceutical-free intervention (Nambudripad D., 2002).

The most severe of all allergic reactions is caused by the IgE antibody and is classified as a Type I Hypersensitivity. When the peanut allergen is introduced into the body via ingestion or inhalation the result is a cascade response resulting in a moderate to severe allergic reaction. The complex reaction that follows involves binding of the allergen to the effector cell by a bridge formed by the IgE antibody. The result is degranulation of the cell, which releases histamines, serine proteases (e.g. tryptase), and other chemicals into the blood stream and tissues. These chemicals are what cause the physiological signs of an allergic reaction such as anaphylaxis. Type I Hypersensitivity produces a rapid rate of response and symptoms are seen within 10 – 60 minutes of exposure (Sheehan, 1997).

The IgE-mediated reaction is the basis of this study. NAET states that it can eliminate IgE-mediated food allergies through non-invasive, holistic methods. Participants in the study were tested for their baseline peanut specific IgE, peanut specific IgG, and tryptase levels prior to undergoing a course of NAET. Following the final treatment each participant was subjected to an oral peanut challenge by the practitioner. Fifteen minutes after the peanut challenge, each participant was again tested for the same antibody and enzyme levels.

A limited number of studies have been carried out with regards to NAET. The majority of these studies were conducted by Nambudripad's Allergy Research Foundation (NARF) and published within the foundation's own non-peer reviewed book: The Journal of NAET Energetics and Complementary Medicine (Lorenzo, 2000). Among these are milk and wheat allergy reduction studies, involving the measurement of IgG, IgM, and IgE antibody levels over a specified period of time. The potential for bias in the conclusions of a private publication enhance the need for further evaluation (Cohen, 2009) (Nambudripad D. S., 2007). Additionally, none of these studies have evaluated at peanut specific antibodies, and NAET is "unproved at this time" (Teuber, 2003). Due to the severity of IgE-mediated

reactions a physiological change within the participant should be detected. The relationship between this peanut allergy treatment and the response seen through antibody and tryptase concentrations may potentially indicate the validity of NAET.

Materials and Methods

Participants aged 6-12 presented with a history of a peanut allergy of any severity and were recruited from local school districts by means of fliers supplied to school nurses. Inclusion criteria were based primarily on a self-reported history of a peanut allergy confirmed previously by scratch tests or allergic reaction. The researchers did not acquire additional documentation of the initial diagnosis of a peanut allergy. For this reason, each participant was evaluated for the presence of an allergy through the results of the pre-NAET blood measurements. Exclusion criteria were based on any condition requiring immunosuppressive drug therapy or previous treatment for food allergy using NAET (Hourihane, 2004). The exclusion criteria were not met by any of the subjects. Participants and their parents were questioned using an entrance survey regarding the severity of their peanut allergy and signed the consent form prior to NAET treatments.

NAET protocol suggests that patients be treated for 15 basic allergens, comprising the first 15 treatments of the participants, and were performed sequentially: BBF (brain-body balance formula), egg, calcium, vitamin C, vitamin B complex, sugar, iron, vitamin A, mineral, salt, grain, yeast, stomach acid, base, and hormone mix (Nambudripad D., 2002). The remaining treatments were comprised of a nut mix including peanuts and walnuts, and then for fear related to peanut exposure. While holding a sealed glass vial of distilled water containing the energy of specified allergen, the subjects laid face down as the practitioner stimulated the nerve roots on both sides of the spine through massage or using a chiropractic impulse instrument. The glass vials were prepared by the NAET foundation and are said to contain the precise energy vibrations that the allergen represents (Nambudripad D., 2002). The same kit was used for each participant. The content and preparation of the vials was not researched further because the exact methodology of NAET was not the primary focus of the study. Following each treatment, the subject remained in the clinic for 15 additional minutes while holding the vial. They were instructed to avoid exposure to the allergen for at least 25 hours post treatment. Each subject underwent 18 NAET treatments within 8 weeks.

During the final treatment the participants underwent a progressive oral peanut challenge. The participants were tested for overall energy balance and for muscle strength while holding the nut mix vial. The initial blood pressure was measured. The participants then held a raw Planters brand peanut,

Arachis hypogaea (Planters, 2011), in one hand and were tested for muscle strength. Following ten minutes of lying and holding the peanut, blood pressure was measured and skin evaluated for any signs of reaction or irritation. The participants were then instructed to moisten their lips and thoroughly rub the peanut on the lips, and were evaluated for ten minutes. Blood pressure was taken and the participant was evaluated for any signs of allergic reaction. The participants were then instructed to consume approximately half of a peanut, and were closely monitored for ten minutes. In cases where any sign of a potential reaction occurred, participants were quickly treated by spinal nerve root stimulation. A final blood pressure measurement was recorded.

Venous blood specimens were collected prior to starting the series of treatments, and within one hour after the oral challenge. All blood samples were collected in serum separator tubes (SST), centrifuged for five minutes at 3800 rpm, the serum frozen at -70˚C, and tested within one month for peanut specific IgG, IgE, and tryptase. Testing was performed at ARUP Laboratories using quantitative ImmunoCAP® and fluorescence immunoassay methods (ARUP Laboratories, 2006 - 2010).

Results

Results for this study are divided into observations and then measurable data. Observations consist of what was observed by the researcher during the progressive oral peanut challenge, and some observations that were reported by the participants within 12 hours. Measurable data consists of the peanut specific IgG, IgE, and tryptase levels measured pre-treatment and then post challenge.

A member of the research team was present for the progressive oral challenge of each participant. For each case blood pressure and pulse remained within normal ranges for their respective age group. Throughout the progressive challenge participants did not express any significant reaction after ingesting the peanut.

As indicated by Table 1, there were no significant changes in serum peanut specific IgE, peanut specific IgG, or tryptase levels in any of the participants. Figure 1 illustrates the lack of significant tryptase elevation following the oral peanut challenge. Reference ranges are given in Table 2 (ARUP Laboratories, 2006 - 2010). In order to eliminate any potential bias due to the limited number of participants, statistical analysis was not performed.

Table 1.

	Peanut IgE (kUA/L)		Peanut IgG (mgA/L)		Tryptase (µg/L)	
Participant	Pre	Post	Pre	Post	Pre	Post
G03 (1)	>100	>100	16.40	15.20	7.45	6.62
G04 (2)	0.16	0.13	7.09	9.35	6.91	6.85
G05 (3)	>100	>100	9.86	11.40	6.02	8.16
G07 (4)	1.78	1.45	3.46	2.79	13.10	13.60
G08 (5)	21.00	22.20	5.63	6.03	5.79	4.52
G09 (6)	0.10	0.10	3.02	4.70	4.04	4.10

Table 2. Reference intervals for measured analysis

Peanut IgE (kUA/L)		**Peanut IgG**
<0.10	No significant level detected	<6.8 mgA/L
0.10 - 0.34	Clinical relevance undetermined	
0.35 - 0.70	Low	
0.71 - 3.50	Moderate	**Tryptase**
3.51 - 17.50	High	0.40 – 10.90 µg/L
>17.51	Very High	

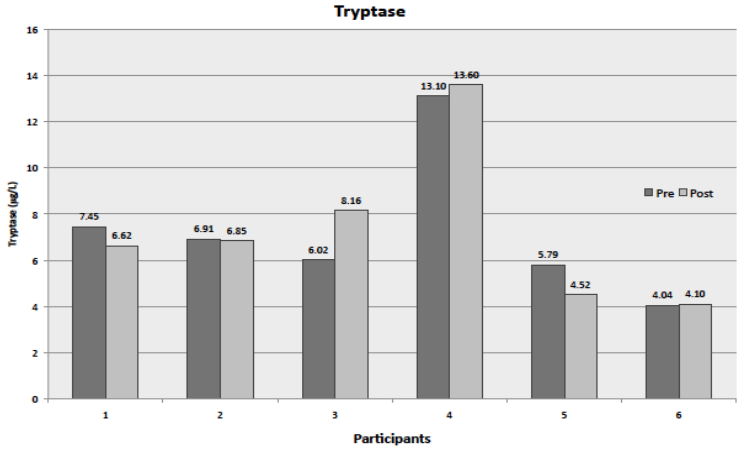

Figure 1. Comparison of the enzyme tryptase pre- and post-NAET treatment

Table 3. Observed symptoms pre-treated and post-oral challenge for each participant.

	Symptoms	
Participant	Pre-treatment	Post-oral challenge
1	Hives, swelling of tongue and throat, anaphylaxis	No Reaction
2	Rash, hives, throat constriction	No Reaction
3	Rash, hives, swelling, difficulty breathing, anaphylaxis	12 hour-delayed hive reaction on abdomen. Treated with inflammation cream.
4	Swelling lips, dry scratchy throat,	No Reaction
5	Hives, swelling, breathing troubles	Slight dyspnea, 25 mg Benadryl given. No further reactions noted.
6	Sore, scratchy, swelling throat, headaches	No Reaction

Discussion

The NAET hypothesis is that the presence of an opposing electromagnetic field from a substance may adversely affect the energy of the human body. A disturbance in the energy pathways may lead to muscle spasms near the spinal nerve roots and cause misalignments of the nervous system, ultimately leading to the symptoms associated with disease or allergic processes. While in the presence of the allergen, stimulation of the spinal nerves relieves these misalignments, and effectively reprograms the energy pathways to tolerate the allergen and prevent reactions (Nambudripad D., 2002).

During an allergic response, the immune system activates and causes symptoms of itching, hives, and anaphylaxis. IgG and IgE antibodies are produced by lymphocytes upon exposure to a foreign antigen, and remain in circulation for rapid subsequent antigen recognition. When IgE antibodies are re-exposed to an allergen, they bind to receptor sites on mast cells. Mast cells are highly concentrated with histamine, heparin, and serine proteases such as tryptase. Binding of IgE antibodies to the mast cell receptors causes degranulation of the chemical contents which may promote and inflammatory reaction leading to anaphylaxis (Lorenzo, 2000) (Payne, 2004) (Sheehan, 1997).

As tryptase is released, additional nearby mast cells are stimulated to activate and release their contents, more cells are attracted to the site of inflammation, and the reaction is quickly amplified. Increased serum tryptase levels are indicative of an immunologically mediated allergic reaction, and cause the clinical manifestations of anaphylactic bronchoconstriction and severe hypotension (Payne, 2004).

Each of the participants had a history of allergic reaction when exposed to peanuts. Self-reported symptoms included immediate onset of nausea, vomiting, itching, hives, and/or anaphylaxis with the necessity of emergency treatment. The serum concentration of peanut specific IgG, peanut specific IgE, and tryptase were analyzed prior to NAET treatment as well as after the oral peanut challenge. No significant change was seen. However, it is important to note that tryptase levels did not elevate. Tryptase concentrations normally maximize within 15-120 minutes, making it an ideal indicator for antibody mediated allergic reaction (Lorenzo, 2000). It would be expected that the concentration of tryptase would drastically increase during an allergic response. With the concentrations of antibodies remaining constant, it seems that an allergic reaction would be inevitable. However, the consistency of tryptase levels indicates that mast cells did not degranulate, perhaps indicating that the antibodies did not bind the mast cell receptor sites (Lorenzo, 2000).

Each participant was closely monitored during the oral peanut challenge. No significant reaction was observed – only minor complaints of temporary oral tingling, pain, anxiety, and nervousness. Blood pressure was monitored throughout the challenge and remained constant. The observations as reported by the parents are included in Table 3. The participants were contacted one week later and asked about further peanut exposure. Each had eaten peanuts in some form, with the worst reactions being moderate hives or nausea. Each reported that reactions had significantly decreased and were no longer severe. No further follow up was performed.

Due to the sensitive nature of the participants and the potential severity of an allergic reaction a null-control group was not included. With previous history of a peanut allergy, the incorporation of a null-control would have placed any control participants at risk for severe complications. Although it may be thought that the treatment mechanism is due to a placebo effect, this is an illogical argument. For a placebo effect to be effective one must believe that treatments or medications are genuinely authentic and beneficial. Some participants have known for several years that peanuts are hazardous to their health and potentially fatal. During the peanut challenge, these fears often overwhelmed confidence in the NAET treatments. Fear of potential consequences was manifest in many of the participants through anxiety, apprehension, and nervousness. With such emotions present, a placebo effect cannot be supported (Barsky, 2002). The mechanism of action of NAET treatments as it relates to immunological activity is not fully understood at this point.

Conclusion

Further research is needed to understand the mechanism of NAET and its relation to the immune system. Based on the data, it is possible that the clinical improvement may have occurred through a mechanism independent of IgE, IgG, and basophils and mast cell responsiveness (Khanolkar, 2011). Additionally, the pediatric population has been shown to manifest allergies that commonly become tolerated as the patient matures (Cochrane, 2009). It is possible that the decreased severity or absence of reaction may be the result of maturation. Future studies should include additional confirmation of an active peanut allergy. Future studies evaluating different routes of the allergic and immune response with an increased participant population, including control groups, could clarify the mechanism of NAET and explain the observed lack of allergic reaction. However, one cannot overlook the absence of significant reaction when compared to previous significant and severe reactions.

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Inhibition of *Clostridium difficile* by Lactic Acid Bacteria

Author: Rachel Lowe, Jed Lowe, & Tarris Webber
Mentor: Craig Oberg

Abstract: In the United States, Clostridium difficile accounts for 15-25% of all antibiotic-associated diarrhea and in 2004, more virulent antibiotic-resistant strains were first identified. This study was done to determine if lactic acid bacteria (LAB) used in dairy products have an inhibitory effect against C. difficile using the agar-flip method. With the difficulty of propagating C. difficile on solid media, 5 media types (SBA SBA with Oxyrase®, MRS agar, MRS agar with Oxyrase®, and EMB media) were tested. Clostridium difficile grew on all media except EMB. Eleven LAB cultures were challenged with C. difficile using the optimized agar flip method. All 11 LAB strains showed inhibition against C. difficile on at least one media type. Since there was no direct contact between C. difficile and the LABs, inhibition may be due to secreted LAB metabolites. Results suggest that specific strains of LAB, perhaps delivered in fermented dairy products, have potential as a treatment for C. difficile infections.

Introduction

Clostridium difficile is an anaerobic, spore-forming bacterium responsible for human diseases ranging from antibiotic-associated diarrhea to pseudomembranous colitis (Anathakrishnan, 2011; Dubberke et al., 2011). *Clostridium difficile* occasionally exists as part of the normal gut flora with complications commonly resulting after treatment with broad-spectrum antibiotics. Antibiotic resistant *Clostridium difficile* strains proliferate over normal gut flora during antibiotic administration (Dubberke et al., 2011). The development of hyper-virulent strains expressing increased toxin production and sporulation rates, along with new surface proteins enhancing the bacteria's ability to bind to the gut more effectively, has recently increased morbidity and mortality rates (Anathakrishnan, 2011).

Cases of *C. difficile* infections (CDI) in the United States are estimated at 3 million annually (Dupont, 2011). In 2010, CDI supplanted methicillin-resistant *Staphylococcus aureus* (MRSA) as the leading hospital-acquired infection with cases of community-acquired CDI in the United States also on the increase (Dubberke et al., 2011). The reoccurrence of CDI in patients after an initial infection is 40% within one month, while patients who experienced more than one CDI have a 60% likelihood of another acute attack (Vedantam and Tillotson, 2011). This high reoccurrence rate has experts suggesting the need for novel therapy development including increased use biotherapeutic techniques when treating CDI (McFarland, 2005).

Lactic acid bacteria (LAB) used as probiotic cultures can supplement the normal human gastrointestinal flora and provide a number of health benefits (Sullivan and Nord, 2009). Several *in vivo* feeding studies have suggested the benefits of LAB administration in treating antibiotic-associated CDI diarrhea (Hickson, et al., 2007; Johnston, et al., 2006). Possible explanations for LAB's therapeutic effect in the treatment of antibiotic-associated CDI include their ability to stimulate the immune response and compete with *C. difficile* for nutrients (Brophy et al., 2005; Sullivan and Nord, 2009). Some LAB strains also produce antimicrobial substances including organic fatty acids, ammonia, hydrogen peroxide, and bacteriocins. Currently, there are very few studies examining the direct *in vitro* inhibition of *C. difficile* by LAB (Cary and Boullata, 2010).

This research involved selecting a growth media on which both LAB cultures and *C. difficile* could grow, and, then, optimizing the agar flip method to screen LAB strains for *in vitro* inhibition of *C. difficile*.

Materials and Methods

Optimizing the Agar Flip Method

Cooked Meat Medium (CMM) broth was used to propagate *C. difficile* (George et al., 1979). Optimal incubation times for *C. difficile* in the CMM broth were determined by measuring turbidity in the inoculated medium, incubated at 37 °C, every 12 h for 96 h. Twelve LAB cultures commonly found in fermented dairy products (Table 1) were grown in either MRS broth or M-17 lactose broth for 24 h at 37°C. All LABs except *Bifidobacterium lactis* demonstrated adequate growth in MRS, which was subsequently used for culture propagation.

Sheep's blood agar (SBA) and MRS media were selected for the agar flip method. To create an anaerobic environment, Oxyrase® (Oxyrase Inc., Mansfield, OH)**,** an enzyme system that removes oxygen from its environment, was added to MRS and SBA media. MRS with Oxyrase® and SBA with Oxyrase® plates were prepared according to manufacturer's specifications. Large diameter MRS agar (MRS-Large) plates (150 mm x 15 mm) were also used if LAB inhibition zones exceeded the diameter of standard Petri plates (100 mm x 15 mm). An attempt was also made to grow *C. difficile* on Eosin Methylene Blue agar (EMB), often used for propagation of anaerobes. Both *C. difficile* and LAB cultures were tested on all five media. Ten, 15, or 20 µl of a LAB MRS broth culture was pipeted in the center of the agar plate to determine which volume would provide an adequate LAB

colony (spot). LAB cultures were incubated at 37 °C, in a gas pak, and checked for growth at 24, 48, and 72 h to optimize spot size.

Inhibition Test - Agar Flip Method

For each media type, 15 ml of each LAB was inoculated on the center of the agar, then plates were incubated at 37°C for 48 hr. The agar in the Petri plate was loosened with an alcohol sterilized spatula and flipped onto the lid of the plate using aseptic technique so the LAB colony is on the bottom of the agar next to the plate lid (Oberg et al., 2009). A lawn of *C. difficile* was swabbed onto the upright side of the agar using aseptic technique. During the first trial, the exposed agar surface was only swabbed in one direction, but for remaining trials, a two pass swabbing method of *C. difficile* was employed with the plate rotated 90° between each swabbing. Inoculated plates were incubated for 72 h at 37°C in a gas pak. Zones of inhibition were measured in mm and recorded.

Results

Optimizing the Agar Flip Method

Cooked Meat Medium broth proved effective to grow *C. difficile*. After 72 h of incubation in CMM broth, the *C. difficile* culture demonstrated limited turbidity and when swabbed on an agar surface confluent growth was not always achieved. Upon swabbing a 96 hr culture, a confluent bacterial lawn was observed leading to more distinct zones of inhibition in the LAB challenge test. Of the media tested, *C. difficile* grew on SBA, SBA with Oxyrase®, MRS, and MRS with Oxyrase®, but more robust *C. difficile* growth cultures was obtained on SBA and SBA with Oxyrase®. No *C. difficile* growth was observed on EMB agar. Lactic acid bacteria (LAB) cultures grew best on MRS and MRS with Oxyrase®; however, growth also occurred on SBA and SBA with Oxyrase®. All LAB cultures grew on EMB; however, due to *C. difficile* results, no further testing was conducted with EMB agar.

To obtain an optimal LAB colony (spot), several LAB inocula volumes were tested. Use of a 15 µl LAB inoculum resulted a 1.5 cm colony (spot) size of after 48 h at 37 °C. After 72 h of incubation, the spot size increased to 3 cm. Upon challenging these larger diameter LAB spots with *C. difficile*, it demonstrated very limited growth; therefore, a 48 h LAB incubation time was used to establish a more measurable zone of inhibition.

Inhibition of Clostridium difficile by Lactic Acid Bacteria

After optimizing the media, inoculum, and incubation time, the agar flip method, used to determine bacterial antagonism, showed *C. difficle* inhibition for a variety of LAB strains (Table 2). Figure 1 shows zones of inhibition for selected LAB cultures and several media types. All 11 LAB cultures demonstrated zones of inhibition on both MRS and MRS with Oxyrase® plates from 2 cm diameter zones to complete inhibition. Larger diameter MRS plates typically didn't offer any advantages over smaller diameter plates (results not shown). Similarly, when regular MRS plates showed complete inhibition of *C. difficile* growth, larger diameter MRS plates also showed complete inhibition indicating the highly inhibitory nature of several LAB strains. Results were more variable when SBA and SBA with Oxyrase® plates were utilized (Table 2). Only *Lactobacillus casei* CLR431 demonstrated a distinct zone of inhibition on the SBA with Oxyrase®. Although not all LAB cultures were tested on SBA plates, *Lactobacillus acidophilus* NEB and L*b. acidophilus* ACD1 demonstrated some inhibition on this media.

Conclusion

Initially, it was necessary to optimize the agar-flip method for use as a rapid screening test for LAB inhibition of *C. difficile.* While the addition of Oxyrase® to several media improved growth of *C. difficile*, results indicate that MRS agar can be utilized for inhibition studies without Oxyrase® addition. This does not preclude the use of Oxyrase® in MRS media since some strains of *C. difficile* may require additional oxygen scavenging to grow more robustly. The optimized agar flip method provided a valuable tool to measure the inhibitory effect of LAB strains on *C. difficile.* Since this method does not involve direct contact between the LAB culture and *C. difficile*, it can be speculated that *C. difficile* inhibition is due to secreted metabolites such as organic acids, hydrogen peroxide, or bacteriocins (Oberg et al., 2009).

Results indicate that specific LAB strains demonstrate an inhibitory effect on *C. difficile* growth and could be used to treat antibiotic-associated CDI diarrhea in a clinical setting. Since there is a high incidence of recurrent infections after initially contracting a CDI, and because long term antibiotic prophylaxis is both expensive and encourages selection of antibiotic resistant organisms, a cheaper and safer alternative may be the feeding of selected LAB cultures to the patient. The agar flip method allows selection of LAB strains with *C. difficile* inhibitory properties that could be used to keep *C. difficile* from reestablishing in the gut. Maintenance of a robust LAB population in the gut using a dairy

delivery system or freeze dried cultures may be a more attractive approach then the current fecal bacteriotherapy or fecal transplant method in use (Bakken, 2009). Other suggestions to help control recurrent CDI include inhibition of spore germination and sensitizing the immune system, which LAB may be able to play some role in accomplishing (McFarland, 2005).

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Table 1. Bacterial Cultures used in the study.

Culture	Genus/Species	Function	Source
LF 23271	*Lb. fermentum*	Probiotic	ATCC
LA NEB	*Lb. acidophilus*	Probiotic	USU
LA5	*Lb. acidophilus*	Probiotic	Chr. Hansen[a]
L10	*Lb. acidophilus*	Probiotic	DSM[b]
BIF 6	*Bf. lactis*	Probiotic	Cargill[c]
LDB 11842	*Lb. delbrueckii* ssp. *bulgaricus*	Probiotic	ATCC
ACD 1	*Lb. acidophilus*	Probiotic	Cargill
CLR 431	*Lb. casei*	Probiotic	Chr. Hansen
F19	*Lb. casei/paracasei*	Probiotic	Medipharm[d]
LAB 7995	*Lb. delbrueckii* ssp. *bulgaricus*	Probiotic	ATCC
PAF	*Pediococcus* sp.	Probiotic	WSU
LBC 1	*Lb. casei/paracasei*	Probiotic	Cargill

[a]Chr. Hansen Inc., Milwaukee, WI.
[b]DSM Food Specialties USA Inc., Eagleville, PA.
[c]Cargill BioActives US, LLC., Waukesha, WI.
[d]Medipharm USA, Des Moines, IA.

Table 2. Size of zones of inhibition measured in millimeters for the different LAB cultures and different medi types

	MRS with OXYRASE	MRS (LARGE PLATES)	MRS	SBA with OXYRASE	SBA
L10	35	35	40	NI	NI
F19	CI	CI	35	NI	
LF 23271	35	CI	CI	SL	
LA NEB	50	100	CI	NI	20
LDB 11842	60	NI	30	SI	
LA5	30	30	30	NI	
ACD 7	30	30	20	NI	15
431	60	50	CI	30	
LAB 7995	40	50	CI	NI	
PAF	40	80	40	NI	EG
LBC 1	40		40	NI	NI

NI – No inhibition
CI – Complete Inhibition (No *C. difficile* growth remaining)
SL – Slight Inhbition
EG – Enhanced *C. difficile* growth over LAB spot

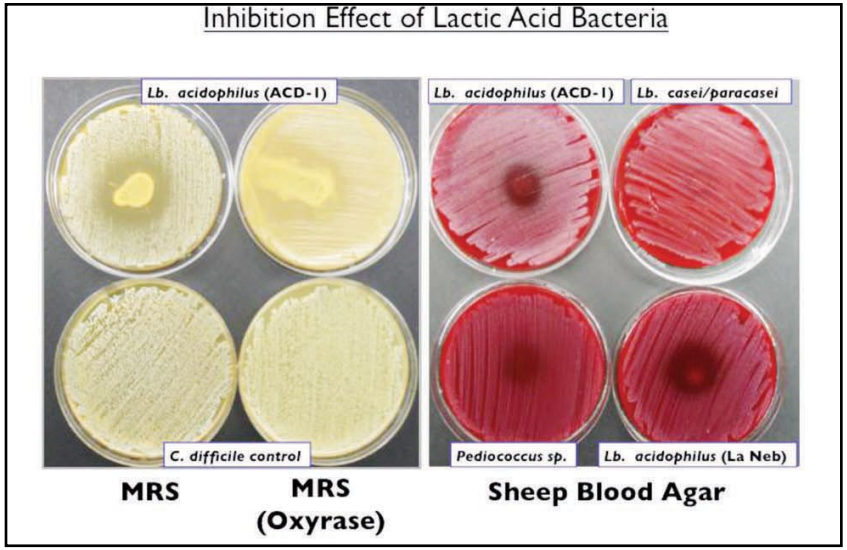

Figure 1. Inhibition of C. difficile by slected lactic acid bacteria using the agar flip method

Novel Marinobacter and a Related Phage Isolated from the Great Salt Lake, UT

Authors: Thomas B. Simon
Mentor: Craig J. Oberg, Michele Culumber & Matthew J. Domek

Abstract: The diversity of microorganisms in the Great Salt Lake (GSL) remains largely unexplored. We have isolated a bacterium related to the genus Marinobacter and a halophilic phage that infects this organism. Water samples from the GSL were diluted, plated on an oligotrophic halophilic medium, and incubated at 30°C for 21 d. A slow-growing colony was selected, cultivated in broth, and identified based on its 16S rRNA gene sequence. Growth was optimum at 30°C and 4-8% NaCl. Phage were enriched by mixing filtered GSL water with the host, and isolated using a soft-agar plaque assay. Phage formed 2-5 mm diameter plaques in 0.8% soft agar. Marinobacter-like organisms have not previously been identified in the GSL and the presence of a specific phage for this isolate suggests the organism is common in GSL water and may be vital to the GSL ecosystem.

Introduction

The Great Salt Lake (GSL) is a hypersaline environment ideal for moderate to extremely halophilic microorganisms (Baxter et al., 2005). A high concentration of nutrients, intense seasonal sunlight exposure, and wind-generated aeration allows the GSL to support a significant number of halophilic microorganisms (Ventosa, 2006). Halophilic microorganisms contribute significantly to the ecological balance in saline lakes since they form the base of the food web, act as decomposers and facilitate nutrient recycling, thus playing a significant role in trophic networks in the lake (Dyall-Smith et al., 2005).

Marinobacter is a genus of euryhaline, *y-Proteobacteria* that primarily inhabits pelagic and benthic marine environments. They have also been isolated from petroleum-field brines, coastal hot springs, hydrothermal vent plumes, saline soils, volcanic basalts, surface seawater, deep seawater and marine snow (Singer et al., 2011). Members of this genus are facultatively anaerobic or strictly aerobic heterotrophs. Some species can grow via hydrocarbon degradation, while others reduce arsenate, oxidize arsenite, and even reduce perchlorate (Gauthier et al., 1992; Handley et al., 2009; Van Ginkel et al., 2010).

Bacteriophages (phage) are viruses that infect bacteria and may be a significant source of bacterial mortality in hypersaline environments (Kauri et al., 1991; Dyall-Smith, 2005). Phages play an important role in recycling nutrient biomass in their environment (Rodriguez-Brito et al., 2010; Suttle, 2007). Phage can also mediate transduction, lysogenic conversion, species successions, and help maintain microbial diversity (Jiang et al., 2004).

In this study, we describe the isolation of *Marinobacter* from the GSL and characterize a bacteriophage that infects this host.

Materials and Methods

Halophilic Medium

Halophilic broth (HB) was used to isolate microorganisms from the GSL. HB contains, per L, 80 g NaCl, 12.5 g MgSO4, 2.5 g casamino acids, 2.5 g yeast extract, 1.25 g protease peptone, 1.5 g trisodium citrate, and 0.1g sodium acetate. Medium was adjusted to pH 7.2 with 1N NaOH prior to sterilization. HB agar (HBA) included 15 g agar per L while HB soft agar (HBSA) had 8 g agar added per L.

Halophile Isolation & 16S rRNA Gene Sequencing

GSL sediment and water were collected from the south arm of the GSL. Water samples were taken 20 cm below the water surface, and sediment from the top 10 cm was collected in water 0.5 m deep. Samples were inoculated on HBA plates and incubated at 30°C for two weeks. As colonies appeared they were transferred to HB. DNA from isolates was extracted using the MoBio UltraClean Microbial DNA Extraction Kit. The 16S rRNA gene was amplified using 27F and 1492R primers (Lane, 1991). DNA was sequenced at the Idaho State University Molecular Research Core Facility, Pocatello, ID. A neighbor-joining phylogentic tree (Figure 1) was prepared in MEGA using the jukes-cantor correction model. Phase contrast images of SA- 51 wet mounts were done on an Olympus BX-41 phase contrast microscope.

Determination of Optimal Salt Concentration for Marinobacter

An 18 hr culture of *Marinobacter* SA-51 was inoculated in triplicate, in 6 ml of HB containing either 0%, 4%, 8%, or 12% w/v NaCl and incubated for 5 d at 30°C. Absorbance ($OD6_{00}$ nm) was measured using a Spec 20 spectrophotometer.

Phage Enrichment

Water samples from the south arm of the GSL were centrifuged at 3000 G for 15 min and filtered through a 0.45 µm filter. One hundred milliliters of HB broth, 8 ml of the GSL filtrate, and 5 ml of *Marinobacter* SA-51 were

combined in a 250 ml flask and incubated at 22°C for 72 h on a shaker. Phage enrichments were centrifuged at 3200 rpm for 10 min then filtered through a 0.45 µm sterile syringe filter.

Isolation of Phage from Enrichment Cultures and Spot Tests

A plaque assay was done by mixing 0.5 ml of the enrichment filtrate with 0.5 ml of host in 4 ml of 0.8% soft agar. This was poured over HBA and allowed to incubate for 24h at 30°C. Agar plugs were removed from the center of plaques using a sterile Pasteur pipette. The plug was transferred to 1 ml of sterile 8% NaCl, vortexed, and incubated for 1 h at 25°C. After 1 h, the plugs and saline were filtered through a 0.45 µm sterile syringe filter. A soft agar overlay was made with the Marinobacter host, then 5 µl of filtrate was spotted on the host lawn to confirm if a phage had been isolated. One phage isolate, TS22, was selected for further study. TS22 was tested against host strain SA-51, SA-52, and several *Salinivibrio* and *Idiomarina* isolates.

One Step Growth Curve

A one step growth curve was conducted to characterize a single cycle of phage replication for TS22. TS22 (0.1 ml) was allowed to absorb to *Marinobacter* SA-51 (0.9 ml) for 5 min at 30°C, after which the infected bacteria were harvested by centrifugation for 1 min at 10,000 rpm. The pellet was resuspended and diluted to prevent further phage adsorption. Samples were taken at timed intervals and titered using the plaque assay. Plaques at each time point were quantified and used to determine the replication time and burst size of TS22.

Results

Isolation of Marinobacter-like organism

From the GSL, an organism from the genus *Marinobacter*, based on 16S rRNA sequencing was isolated (Figure 1). Phase contrast images of SA-51 wet mounts exhibited cell morphology characteristic of this genus (Figure 2). Growth for SA-51 was optimal at 4-8% NaCl with minimal growth observed at 0% NaCl, characteristic of a euryhalophile (Figure 3).

Isolation of a Marinobacter Bacteriophage TS22

Plaques of TS22 varied in size from 2-5 mm and appeared after 48 hr at 30°C (Figure 4A). Although plaque size varied in original isolation plates, re-purification of individual plaques showed consistent plaque sizes (Figure 4B). Plaque size variation could be due to the growth phase of host cells at the time of infection or to the ionic strength of the medium (Kukkaro and Bamford, 2009). Spot tests confirmed that TS22 does not infect SA-52 or *Idiomarina* and *Salinivibrio*. Phage that infect these genera do not infect SA-51 (data not shown).

One Step Growth Curve

In the one step growth curve, the latent period is the time from phage DNA injection to the release of mature phage, and the burst size is the average number of mature phage particles released by lysis of a single cell. Burst size is calculated by dividing the phage concentration at the end of the growth cycle by the initial concentration. Results showed TS22 produced a burst size of 65 PFUs per cell and that the phage replication cycle took 40 min with a latent period of 10 min (Figure 5).

Conclusion

Marinobacter-like bacteria have not been previously reported in the GSL (Baxter et al., 2005). We isolated two members of this genus from GSL water. One isolate, SA-51 had a wide salt tolerance exhibiting growth from 4-12% NaCl with optimum growth at 4-8% NaCl, consistent with the salt concentrations in the GSL south arm. Since south arm salinity varies seasonally, a wide salt tolerance would enable these organisms to survive in a changing environment. Organisms from the *Marinobacter* clade are commonly found in moderately saline environments, where they can account for a large proportion of the microbial community (Singer et al. 2011). Some species of *Marinobacter* are capable of hydrocarbon degradation (Gauthier, et al., 1992) and the GSL contains numerous natural oil seeps that could supply *Marinobacter* with these nutrients. The metabolic capabilities of SA-51 should be evaluated as it could be useful for bioremediation of moderately halophilic ecosystems contaminated with hydrocarbons.

The presence of a specific bacteriophage for isolate SA-51 suggests this organism is also relatively abundant in GSL water. We believe this organism has not been isolated previously because it requires a low nutrient medium and longer incubation time for the initial colonies to appear. Quickly growing organisms (e.g. *Salinivibrio, Idiomarinia*) may outcompete *Marinobacter* on high nutrient media. Using oligotrophic media and extended incubation strategies allowed isolation of organisms not previously identified in the GSL.

TS22 replicates quickly (40 min) with mature phage particles produced after 10 min. TS22 showed a relatively small burst size (65 PFUs). NS01, a phage that infects *Salinivibrio costicola* isolated from the GSL, showed a burst size of 100 PFUs (Savage et al., 2009). More work is needed to understand the significance of burst size with regards to impact on modulation of bacterial populations.

Bacteriophage, such as TS22, are likely important for controlling the

population sizes of halophilic microorganisms in the GSL (Rodriguez-Brito, et al., 2010). Other than brine shrimp, GSL microorganisms have few predators so phage are important for recycling nutrients stored in the microbial biomass. Our experiments demonstrate that SA-51 has a unique phage and does not infect a closely related isolate, SA-52. This could indicate that this phage is involved with controlling the population density of SA-51.

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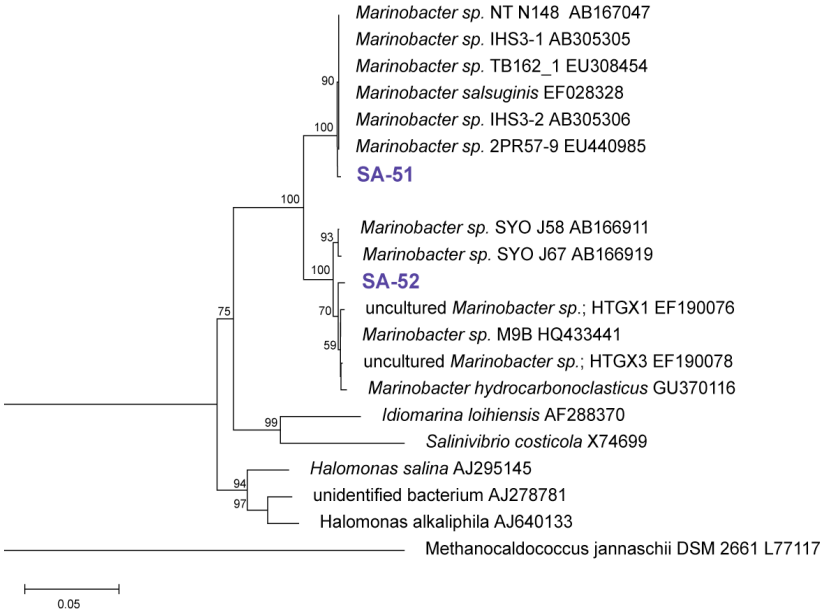

Figure 1. *Phylogenetic Tree for Marinobacter SA-51 and SA-52 isolated from the GSL. Tree nodes with greater than 50% bootstrap replicates are indicated on the tree (100 bootstrap replicates were performed).*

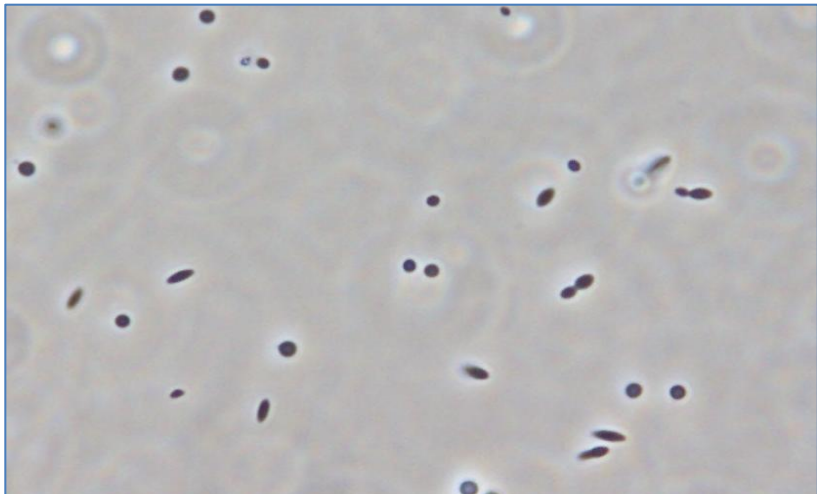

Figure 2. *Phase contrast images of SA-51 (1000x). SA-51 is a motile rod. Some cells were club-shaped and cell arrangement was as single or diplobacilli.*

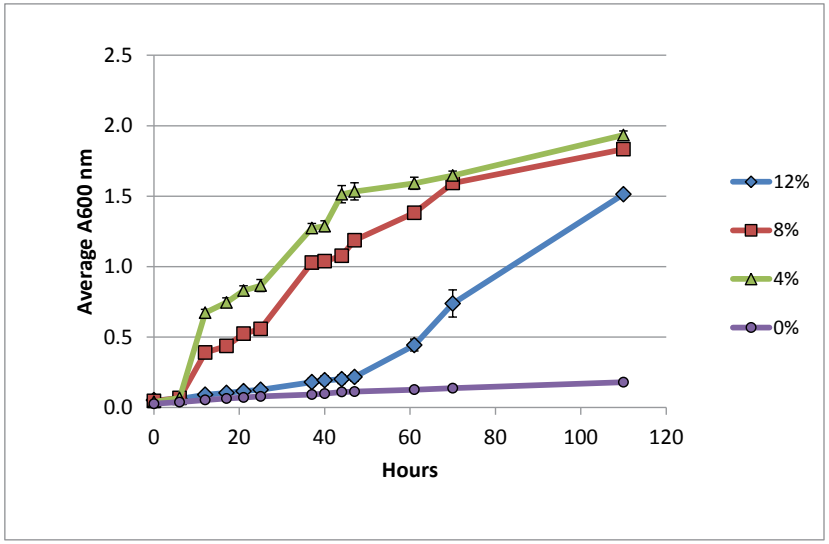

Figure 3. *Growth Marinobacter SA-51 at different NaCl concentrations*

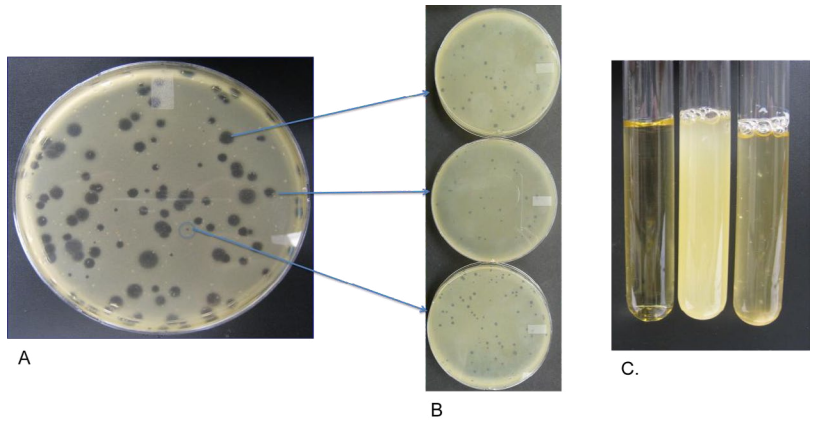

Figure 4. *A. Plaque morphology for TS22 on Marinobacter SA-51. B. Secondary isolation of large, medium and small plaques. C. Isolate SA-51 demonstrating clearing due to phage infection: tube 1 uninoculated HB; tube 2 non-infected SA51, tube 3 SA-51 infected with phage TS22.*

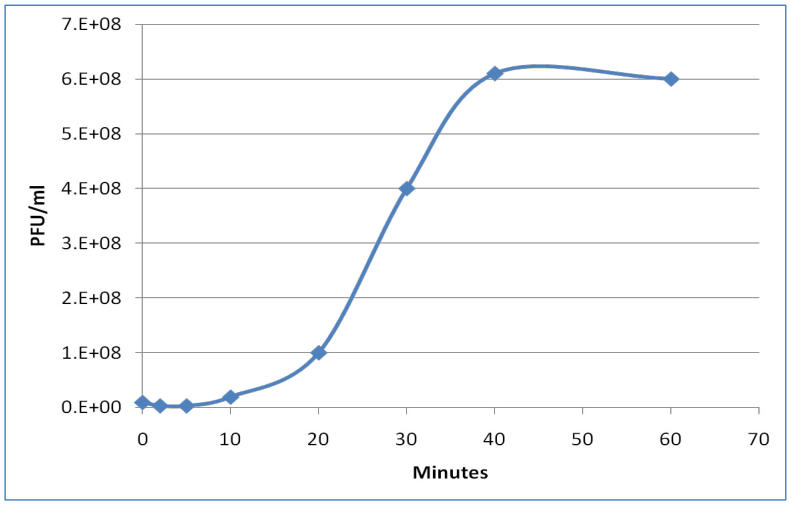

Figure 5. *One Step Growth Curve for Phage TS22.w*

Similar Infection with No Relation: Genome Analysis of Two Novel Halophage

Author: Preston Kerr
Mentor: Matthew Domek

Abstract: Bacteriophage are viruses that infect bacteria and play a significant role in keeping bacterial populations in balance. In previous studies two novel halophage, NS01 and CW02 were discovered in the Great Salt Lake (GSL). The phage were initially believed to be related because they both infect strains of Salinivibrio costicola that are very similar, however analysis of the genomes reveal that the phage are not related. For the sequenced genomes of NS01 and CW02 ORFs were found using DNA Master and a combination of Genemark and Glimmer. A total of 75 ORFs were found for CW02 and 105 ORFs for NS01. Of these, 17 ORFs for CW02 and 19 ORFs for NS01 were annotated with strong support. GC% differed slightly among both genomes. Seven tRNA's were found in NS01, and one tRNA was found in CW02. A DNA polymerase was found in CW02 and two RNA polymerases in NS01. The two capsid proteins of NS01 were compared against the capsid protein of CW02 using BLASTp and shown to be very different as well. CW02 and NS01 were compared using Needleman-Wunsch and shown to be quite different. A BLASTn local alignment of CW02 against NS01 and several other phage also shows the phage to be quite different from one another. Sequence alignment results, differences in GC%, genome lengths, polymerases found, the number of tRNA's, and the overall layout of the genomes suggests that the phage are not very similar. This study provides some insight into phage in the GSL that is lacking in the current literature. The results of this study provide a basis of comparison in future GSL studies.

Introduction

The Great Salt Lake (GSL) is a hyper-saline environment inhabited by a variety of halophilic Archaea, Bacteria, and other microorganisms. Bacteriophage are viruses that prey on bacteria and are believed to play a significant role in controlling bacterial populations. Not much is known about bacteriophage from the GSL, however, in prior studies, two phage that infect different strains of *Salinivibri costicola* were isolated from the GSL. The phage, NS01 and CW02, were characterized as icosahedral in shape and had double stranded DNA genomes (Savage et al. 2009, Culumber et al. 2011). Subsequently, the genomes of the phage have been sequenced. Our hypothesis was that NS01 and CW02 are similar phage because of the and similarities between the host strains. *S. costicola* S39 is the host for NS01 *S. costicola* S50 is the host for CW02. Although these strains are very closely related based on their 16S rRNA gene sequences, the phage are specific for their hosts. In this study we used bioinformatics to annotate the CW02 and NS01 genomes and attempted to determine if NS01 and CW02 were related phage.

Methods

Source cultures and phage from the GSL

S. costicola strains S39 and S50 and their associated phage were isolated in previous studies, and are maintained in the Weber State University Halophile Culture Collection (Savage et al. 2009). The DNA from NS01 and CW02 was isolated in a previous study (Culumber et al. 2011). DNA genome sequencing was provided by the BYU DNA Sequencing Center using a 454 pyrosequencer.

Genome Annotation

The sequences were annotated using the University of Pittsburgh Annotation Protocol with modifications (University of Pittsburgh, 2010) and the program DNA Master. Open reading frames (ORFs) are gene segments with a high probability of coding for a protein. ORFs were found using a combination of Genemark and Glimmer (Besemer, Borodovsky 2005, Delcher, et al. 1999). The identified ORFs were assigned functions through BLASTp analysis on the National Center for Bioinformatics (NCBI) servers, using algorithms Blossum 45, 65, 80 and Pam 30 and 70 (Lipman et al 1997). EMBL SMART genomic Scans and InterproScans were used to search for transmembrane regions, unique 3D structures, motifs, and conserved regions (Bork et al. 1998, Zdobnov, Apweiler 2001). Automated Protein Function Prediction (PFP) and Extended Similarity Group Scans (hosted on servers at Kihara Bioinformatics Laboratory at Purdue) were used as further support for assigned functions. The program tRNA Scan SE was used to search for tRNA genes in the genomes (Schattner, Brooks, Lowe 2005). Capsid proteins of NS01 and CW02 were compared to each other, and several other bacteriophage using a local BLASTp. A Needleman-Wunsch global alignment was used to determine overall sequence similarity between CW02 and NS01 (Needleman S, D. Wunsch 1970). A BLASTn local alignment of CW02 against several phage, including NS01 was run on NCBI servers using BLASTn.

Results

A summary of the morphology of NS01 and CW02 and the general characteristics of their genomes can be found in Table 1. The genomes had some significant differences including size, GC content, number of ORFs and the number of tRNAs.

The ORFs annotated for each genome are listed in Table 2. The ORFs were found using a combination of Glimmer and Genemark scans run within DNA master. Web versions of Glimmer and Genemark were run to confirm results. A majority of ORFs when run against NCBI servers using BLASTp had no significant matches. Of 75 ORFS for CW02 and 105 for NS01, only 17 and 19 ORFs respectively, could be strongly annotated. These ORFs had low E-values, contained conserved sequences, and/or were supported by clustering of similar products. A low e-value means that the probability of the results of the BLASTp being due to chance alone is very low. Conserved sequence regions often code for the same products across species, genus, and sometimes domains. More related species will contain a larger number of similar and conserved sequences. Therefore conserved sequences found were considered strong evidence for relatedness. Information gained from the programs EMBL SMART Genomic Scans and InterproScans confirmed protein motifs and conserved sequences found by BLASTp. Automated Protein Function Prediction (PFP) and Extended Similarity Group Scans helped confirm the functions assigned to ORFs.

The CW02 capsid protein (ORF 10) was aligned against both NS01 capsid proteins (ORFs 61 and 73) using BLASTp. The results are summarized in Table 3. A Needleman-Wunsch global alignment was performed on NS01 and CW02. When NS01 and CW02 were aligned against each other a NW score of -52191 with 29% of the sequence gapped and a 47% max identity was yielded. As a comparison, a separate alignment was performed on mycobacteria phage “Eagle” and “Peaches”. These two phage yielded a score of 91,375 and a 96% max identity with only 1% of the sequence gapped. A BLASTn local alignment was performed between CW02, NS01, and several phage. The results are listed in Table 4.

Discussion

Initially it was believed that the genomes of NS01 and CW02 would be similar. Each halophage infected distinct, but closely related, strains of S. costicola isolated from the GSL. DNA was linear double stranded, and the capsid was Icosahedra. This is apparently where the similarities end. The overall characteristics of the genomes were found to be very different. The genome of NS01

is almost 17 kbp larger than CW02 with about 30 more ORFs. The GC% of the genomes is also slightly different, and 6 more tRNA’s were found in NS01 (Table 1). After annotation, patterns appeared in the genome organization (Table 2). CW02 has structural proteins encoded on the + strand, and replication proteins on the - strand. NS01 has a similar layout, but structural proteins are found on the – strand, and replication proteins on the + strand.

Many ORFs could not be annotated. The ORFs found coded for either structural proteins or enzymes involved with nucleic acid synthesis. Capsid ORFS were found in each phage and compared. The CW02 capsid (ORF 10) was aligned using BLASTp against both capsid proteins of NS01 (Table 3). The E-values were both high (> 0) and the max scores were low. A high E-value may mean that the results occurred due to chance alone. These results show that the capsid proteins are not conserved between these phage.

Different types of polymerases were found in each phage (RNA polymerase in NS01 and DNA polymerase in CW02). Because polymerases are often highly conserved, especially among species, it is expected that if RNA polymerase were present in NS01, it would be present in CW02. The same goes for DNA polymerase. This provides support that the phage are not closely related to another.

Overall similarity of the genomes was tested with a Needleman-Wunsch (NW) global alignment and a BLASTn local alignment (Table 4). A NW alignment was also performed on two mycobacteria phage “Eagle” and “Peaches” for reference. They are extremely similar, and have a difference of only 100 bp. A NW alignment of these two phage gives a very high max score and max identity. Comparing this to the low score of CW02 and NS01 show us that CW02 and NS01 are not very similar. When NS01, CW02, and several phage genomes were compared with a BLASTn, all showed low alignment scores. The higher scores signify that CW02 is in fact more similar to Bacteriophage PA11 or Roseobacter Phage, than it is to NS01 (Table 4). However, the scores are still low, showing that CW02 is not very similar to Bacteriophage PA11 and Roseobacter Phage either.

Conclusion

Although they infect similar hosts, halophage NS01 and CW02 do not seem to be closely related. NW and BLASTn alignment results, differences in GC content, genome lengths, tRNA’s, and the overall layout of the genomes suggests that the phage are not very similar. The absence of the same types of polymerases in each phage genome strengthens this conclusion. These results provide information on halophage located in the GSL that is currently lacking in the literature. This work provides future annotation studies with

with a basis to compare against.

Acknowledgments

R. Hoggan and B. Dahl were responsible for the DNA extraction of NS01 and CW02. P. Shen and D. Bishop were responsible for the sequencing of NS01 and CW02. I would like to thank M. Domek and M. Culumber for the donation of their time, expertise, and lab to the project.

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Appendix

Table 1. Summary of CW02 and NS01

CW02 vs. NS01		
	CW02	NS01
tRNA's	1	7
BP	49396	66254
GC%	47.67	46.32
ORFs	75	105
Morphology	Linear DS DNA	Linear DS DNA
Capsid	Icosahedra	Icosahedra
Host	*Salinivibrio costicola*	*Salinivibrio costicola*
Location Isolated	GSL	GSL

Table 2. Results from a blastp of NS01 and CW02 capsid proteins

CW02 Capsid Vs. NS01 Capsid proteins using Blastp				
	Max score	**Query Coverage**	**E value**	**Algorithm**
CW02 Capsid Protein vs. NS01 Capsid Protein (ORF 61)	11.1	6%	3.9	Blos 45
CW02 Capsid Protein vs. Capsid Protein (ORF 73)	13.7	83%	2.1	Blos 45

Table 3. Results from a blastn of Cw02 againts NS01 and several other phages

Blastn Local Alignment for CW02					
	Max Score	**Total Score**	**Max Identity**	**E Value**	**Query Coverage**
Bacteriophage PA11	650	1916	78%	0	13%
Roseobacter Phage S1O1	159	460	73%	1 E-33	2%
Staphylococcus Phage SA1	102	152	87%	3.00E-16	0%
NS01	89.7	999	100%	3.00E-18	2%
Serratia Proteamaculans 568	86	86	84%	2.00E-11	0%

Table 4. Results from a BLASTn of CW02 againts NS01 and several other phage

CW02					
Product	**ORF #**	**Strand**	**Start**	**Stop**	**Length**
Head/tail connector	5	+	4850	6628	1779
Head Fiber	10	+	9268	9612	345
Tail needle	16	+	13511	17752	4242
Endonuclease	20	+	20491	20799	309
Collagen-Like protein	21	+	20803	22629	1827
tRNA nucleotidyltransferase	23	–	22840	23415	576
Polymerase, 5'3' Exonuclease	32	–	27854	28063	210
Hypothetical Conserved Phage Protein	33	–	28073	29962	1890
DNA Polymerase	35	–	30170	30655	486
Sigma Factor	37	–	31147	32745	1599
Primase/Helicase	40	+	33360	33740	381
Uncharacterized Conserved Phage Protein	42	+	33959	34192	234
ATP Grasp Protein	43	+	34189	34410	222
Amido Ligase	50	+	37439	37672	234
GATase	51	+	37681	38484	804
Cell Wall Hydrolase	63	+	45120	45596	477
Short Chain Dehydrogenase	64	+	45593	45769	177
tRNA-Arg(tct)		+	46001	46077	77

Table 4. Continued

NS01					
Product	**ORF #**	**Strand**	**Start**	**Stop**	**Length**
RNA Polymerase	1	+	67	729	663
Radical Sam protein	44	+	25506	26567	1062
Thymidine Kinase	46	+	26773	27318	546
Hydrolase	48	+	27651	28175	525
Capsid protein	61	–	38914	39990	1077
Tail fiber	65	–	42528	43340	813
Conserved Phage Protein	69	–	45864	46094	231
Collagen like protein	72	–	47414	49174	1761
Capsid protein	73	–	49174	49467	294
Conserved Phage Protein	75	–	50672	51244	573
Terminase	77	–	53656	55632	1977
Terminase	80	–	57131	57535	405
RNA Polymerase	99	+	65147	66253	1107
tRNA-Pro(tgg)		+	56103	56181	79
tRNA-Trp(cca)		+	56305	56380	76
tRNA-Tyr(gta)		+	56385	56475	91
tRNA-Ile(gat)		+	56482	56560	79
tRNA-Asn(gtt)		+	56566	56640	75
tRNA-Glu(ttc)		+	56720	56797	78
tRNA-Arg(tct)		+	56836	56914	79

The Effects of Chlorpyrifos on the Growth and Viability of *Aspergillus flavus*

Author: Rebecca Peters
Mentor: Barbara Trask

Abstract: Because the carcinogenic mold Aspergillus (A.) flavus is so prolific and economically destructive on such a wide variety of crops, it is important to identify agricultural processes that may impact its growth or survival rate, so that possible methods for its control can be designed. This experiment aimed to determine whether chlorpyrifos, the active ingredient in many pesticides used in areas of high A. flavus contamination, has any effect on its growth. Aspergillus flavus was grown in Petri plates on potato agar medium containing gradient doses of chlorpyrifos. Plates at each dose were harvested daily for five days and total RNA was harvested by phenol/chloroform extraction. mRNAs were reverse-transcribed and cDNAs for two genes associated with fungal growth and viability, ATP synthase and chitin synthase, were amplified by polymerase chain reaction (PCR). Reverse-transcribed PCR products were analyzed for quantification using Image J software. Preliminary data shows that chitin synthase mRNA expression was diminished in parallel with increasing pesticide concentration, suggesting that growth of A. flavus was inhibited. However, ATP synthase mRNA expression was increased at higher doses of chlorpyrifos, indicating that the fungus is able to thrive in these conditions.

Introduction

Like most soil-based fungi, *Aspergillus flavus* is an aerobic, mostly asexual saprophyte that thrives in hot, moist conditions (Bennett, 2010). The fungus occurs naturally on groundnuts, treenuts, grains, and spices, and grows with near impunity in equatorial areas that do not have the $750/acre required to prevent it from contaminating crops, such as South Asia, Central America, and the majority of the African continent (Bennett, 2010; USDA, 2011). In many of these areas, food shortages and famine give farmers and villagers little choice but to eat contaminated crops, regardless of the amount of spoilage, which leads to illness, cirrhosis, and early death (Abbas et al., 2005).

Unlike most fungi, *A. flavus* produces a potent mycotoxin that is ranked among the most deadly organic carcinogens on the planet – aflatoxin (JECFA, 1998). A secondary metabolite of *Aspergillus flavus,* aflatoxin is a confirmed mutagen, teratogen, Group 1 carcinogen, and toxin with both

acute and chronic effects (JECFA 1998). It frequently survives most food preparation techniques, and will remain on contaminated foodstuffs long after the death of the mold, unless thoroughly ammoniated (Abbas et al., 2005). In the United States alone, *A. flavus* causes well over $1 billion in losses from crop damage and regulation requirements each year. (Abbas et al., 2005) When aflatoxin levels in crops exceed the FDA's limit of 20 ppb, the crop can either be ammoniated or sold at a discount for use as animal feed (Finley, Robinson, & Armstrong, 1992). Because of this, aflatoxin has been found in many consumables produced by animals (milk, eggs, etc.) fed with contaminated crops. The FDA's determined acceptable level of aflatoxin in these foodstuffs is 0.5 ppb (Finley, Robinson, & Armstrong, 1992).

Due to its staggering effect on human welfare, the genus *Aspergillus* has been extensively studied (Bennett, 2010). Scientists internationally have studied many aspects of most *Aspergillus* species and, to date, the genomes of 15 different *Aspergillus* species have been sequenced, including that of *A. flavus* (Bennett, 2010).

Pesticides containing chlorpyrifos (e.g., Lorsban™) are commonly employed in geographic areas such as the southern United States, with climates conducive to the growth of *A. flavus*. The effect that this commonly-used chemical has on the growth and viability of *A. flavus* has never been specifically studied before. This experiment aims to add to the expanding knowledge of these fungi by illuminating the relationship between *A. flavus* and the active ingredient in many pesticides, chlorpyrifos.

Materials and Methods

Chlorpyrifos concentrations used in this study were calculated by extrapolating from modern pesticide use recommendations (dowagro.com/usag/prod/034), and closely mirror those used in similar experiments with different fungi (Trappe, Molina, & Castellano, 1984). Chlorpyrifos was obtained from Sigma Chemical Company and *A. flavus* was purchased as a freeze-dried culture from the America Type Culture Collection (ATCC).

Aspergillus flavus was resuspended as detailed by the ATCC. The fungus was grown on hand-prepared potato dextrose agar . Following sterilization by autoclaving, growth agar was allowed to cool to 50°C before the addition of chlorpyrifos at the following concentrations: 200µg/ml, 100µg/ml, 50µg/ml and 0µg/ml. Each molten medium was then poured into 60mm Petri plates and allowed to cool and solidify. 12,000 kDa molecular weight cut off (MWCO) dialysis tubing (Corning) was wetted in sterilized water and was subsequently cut into 60mm diameter circles that were then placed onto each of the growth plates to minimize fungal growth into the agar (to simplify isolation). The use of a high MWCO dialysis tubing, however,

allowed for the diffusion of nutrients so that the fungus was able to grow as usual. Each of 40 plates, ten at each of the above chlorpyrifos concentrations, was then inoculated with 20µl of the resuspended *A. flavus*.

Each day for five days two random plates were selected from each of the four doses, and *A. flavus* (adhered to the dialysis membrane) was peeled off the plates. Holding the membrane with sterilized forceps, fungus was removed by rinsing it several times with 1-5 ml of Trizol (Invitrogen). Trizol fungal suspensions were homogenized using a Tissue Terror (Fisher Scientific). Trizol homogenates were stored at -80°C until all samples had been collected. Total RNA was isolated from the Trizol homogenates following the manufacturer's protocol. RNA pellets were air dried, resuspended in DEPC-treated water and quantified by spectrophotometry at 260nm.

To perform the reverse-transcription reaction, 1µl of total RNA from each sample was diluted to 13µl in water and buffer, and was heated for 10 minutes at 65°C. Samples were put immediately on ice to cool following this heat denaturation. Additional 5x GoTaq buffer (Promega), plus MMLV reverse-transcriptase enzyme (Promega), RNase OUT (Invitrogen), dNTPs (Invitrogen), and random primers (Invitrogen) were added to produce a final reverse-transcription reaction volume of 20 µls. Reverse-transcription (RT) proceeded for 40 minutes at 42°, followed by 5 minutes at 95°C to deactivate the enzyme. Samples were placed on ice, and each 20 µl reaction was divided into two tubes of equal volume so that identical reverse-transcription reaction products from each sample could be subjected to PCR amplification. Additional water, 5X buffer and GoTaq polymerase (Promega) was added to each ½ RT reaction to yield a final volume of 48 µls. Two µls of a forward and reverse primer pair mixture (for either chitin synthase or for ATP synthase) were added to each sample. PCR was performed using an Indy Thermocycler (Idaho Technologies) One percent agarose gel electrophoresis confirmed that products of the expected sizes were obtained. Electronic images generated using a ChemiDoc analyzer and Quanity One software (Bio-Rad) were analyzed using Image J software to quantify the PCR products for each sample.

Results

Of the 40 plates, only 25 (8 controls, 5 plates at 50µg/ml, 6 at 100µg/ml, and 6 at 200µg/ml) yielded sufficient quantities of total RNA. The results show that, under control conditions, *A. flavus* produced similar amounts of both ATP synthase and chitin synthase. However, when exposed to chlorpyrifos, higher concentrations of the pesticide caused the fungus to favor expression of ATP synthase over chitin synthase. Less chitin synthase was produced than in the control group, while levels of ATP synthase

increased in comparison to the control group. A statistical analysis was performed on the production of both chitin synthase and ATP synthase. Hypothesis tests on the mean of the experimental groups were conducted in each case as they compared to the means of the control groups. The test statistic was assumed to follow the student-t distribution with five degrees of freedom. The analysis showed that the decrease in chitin synthase and the increase in ATP synthase were both statistically significant at the $\alpha = .025$ level.

Discussion

This experiment shows that a shift in mRNA expression occurs when *A. flavus* is exposed to variable concentrations of chlorpyrifos. The implications of these preliminary results, while largely conjecture, are potentially quite powerful. Although other studies (Yin, Chang, 1998), (Fakhoury, Woloshuk, 2001) confirm that certain herbs and spices can restrict the growth of *A. flavus*, the relationship of the mold with most pesticides is still poorly understood. In addition, Lorsban, the brand name of chlorpyrifos, advertises effectiveness against other fungi, such as white mold, even though the pesticide itself acts as a cholinesterase inhibitor, and fungi do not use cholinesterase (dowagro.com/prod/034). Consequently, a more complete understanding of the chemical's relationship with fungi could possibly be beneficial to other chemicals that act upon the nervous system and also come into contact with molds.

The experiment reveals that growth of *A. flavus* is significantly affected by the pesticide chlorpyrifos. Chitin synthase is used to produce chitin, the major component of fungal cell walls. Therefore, the amount of chitin synthase produced in a fungal culture is directly related to the number of cells produced and, by extension, the size of the fungal colony. Because chitin production was reduced when *A. flavus* was exposed chlorpyrifos, it was inferred that the colony began to put less effort into cell division, and more effort into energy production. In fact, chitin production by *A. flavus* decreased with even minimal amounts of chlorpyrifos in the environment. Because the enzyme chitin synthase is required for cell wall production, this result suggests that chlorpyrifos inhibited cell division, as it is expected that colonial expansion would necessitate cell wall production. In contrast, ATP production increased dramatically for cultures grown in higher doses of chlorpyrifos. Because ATP production is dependent upon ATP synthase activity, these results suggest that, although *A. flavus* failed to reproduce in the presence of pesticide, the fungus continued to be metabolically active even in chlorpyrifos-rich environments, and that the effort was instead directed toward ATP production. Because intracellular ATP concentrations

were not directly measured, an increase in this energy source has not yet been verified. If such an increase does prove to result from chlorpyrifos exposure, a specific use of any additional ATP produced is still unknown as well.

While unverified, it is easy to speculate that a fungal colony, sensing a threat from chlorpyrifos in the environment, might begin producing increased levels of aflatoxin in an effort to destroy competitors and thereby make conditions more favorable for growth. An alternative hypothesis is that environmental conditions in the presence of chlorpyrifos might be sufficiently bad such that extra ATP would be required for the fungus to function and metabolize at a regular rate. Either possibility is currently conjecture, as the ATP requirements of a healthy *A. flavus* sample are unknown, and aflatoxin levels were not quantified during this experiment.

The fact that the *A. flavus* can survive in chlorpyrifos suggests that the organism can possibly enjoy reduced competition from others that chlorpyrifos effectively inhibits. Further tests employing multiple species would be required to support this. Additional tests in this area should be performed such that the nature of the *Aspergillus flavus*/chlorpyrifos relationship might be better understood.

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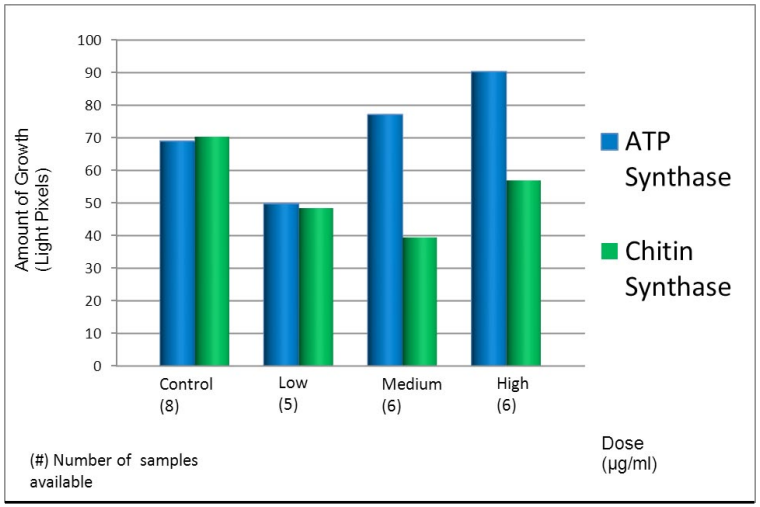

Figure 1.

The Effect of Stevia on Four Intestinal Flora

Authors: Allison Scheel, Teresa Reyes & Rebecca Fox
Mentor: Scott Wright

Abstract: With the recent influx of artificial sugars into the market, it is important to understand what effect these chemicals have on the human intestinal tract. Stevia is a sugar substitute that has recently been released. Stevia is derived from the Stevia rebiana plant. The objective of this research was to determine if stevia had any significant effect on normal intestinal flora, compared with regular table sugar. This was accomplished by mixing Escherichia coli, Lactobacillis acidophilus, Bacteriodes fragilis, and Bifidobacterium bifidum cultures with varied concentrations of stevia and table sugar. We then inoculated growth media with the sugar-bacteria mixtures and the bacterial growth was quantified and compared using t-tests to determine significance. After collecting and analyzing the data, it was determined that stevia had no significant effect on the growth of E. coli, L. acidophilus, or B. fragilis compared with table sugar. The B. bifidum culture did not grow in a standard nutrient broth, and was therefore excluded from the determination of significance.

Introduction

Since saccharine, the chemical in Sweet 'n' Low™, was accidentally invented in 1879, manufacturers have been using sweet tasting chemicals to replace table sugar in a variety of foods (Science Channel, 2011). Artificial sugars are an attractive alternative for individuals looking to limit their calorie intake. They also allow people with medical conditions, such as diabetes, which prevent them from eating large quantities of sucrose, to enjoy foods they would otherwise have to avoid. A variety of artificial sugars, including saccharine, aspartame, sucralose, and stevia, are now available to the public under brand names such as Sweet 'n' Low™, NutraSweet™, Splenda™, and Truvia™, respectively. Given the promise of all the taste with none of the negative effects associated with table sugar, an increasing number of individuals are replacing sugar with artificial alternatives (Mattes & Popkin, 2009). As artificial sugars are composed of chemical compounds humans may not normally consume, it is possible that they may have unexpected effects on the people who use them.

Splenda™, an artificial sugar which utilizes sucralose, was released into the U.S. market in 1999. Many individuals questioned the safety of this new sweetener, and as a result, several studies have been conducted on the effects of Splenda™. One of these studies investigated the effects of Splenda™ on the intestinal flora of rats. The study demonstrated that Splenda™ reduced the intestinal flora of rats by up to 50 percent (Abou-Donia, El-Masry, Abdel-Rahman, McLendon, & Schiffman, 2008).

Another artificial sweetener, stevia, was approved for use in the U.S. by the Food and Drug Administration (FDA) in 2008 (LLC, 2008). This research sought to determine if stevia had any significant effect on the normal intestinal flora of humans. Four intestinal bacterial species were evaluated: *Escherichia coli*, *Lactobacillis acidophilus*, *Bacteriodes fragilis*, and *Bifidobacterium bifidum*. These bacteria are very prevalent in the intestinal tract and, along with several others, and were analyzed in the study on Splenda™ (Abou-Donia et al., 2008).

Materials and Methods

The design of this study was approved and funded by Weber State University's Office of Undergraduate Research. The following strains of bacteria were purchased and grown in either Columbia Nutrient Broth (for aerobic organisms) or Thioglycolate Broth (for anaerobic organisms): *B. bifidum* ATCC ®11863, E. coli ATCC®, L. acidophilus ATCC®314, and *B. fragilis* ATCC®25285. Gas Pak EZ Anaerobe gas packs and anaerobic indicator strips were used for *B. fragilis*. 1 uL and 10 uL calibrated urine loops were used to standardize the amount of inoculate.

These bacteria were incubated in nutrient broths, infused with either stevia or table sugar for a period of 24 hours, then inoculated onto Sheep Blood Agar (SBA). Individual colonies were counted to determine if there was any difference in growth between the organisms which were incubated in table sugar and those which were incubated in stevia. This project hoped to determine if there was a significant effect due to stevia on normal intestinal flora.

Three concentrations were used for the sugar (C&H pure Cane Granulated White Sugar) and stevia (Kel Pure Stevia) solutions. All concentrations were based on the estimated daily intake (EDI) of stevia which was determined by the FDA to be 5.2 mg/kg of body weight (LLC, 2008). We used a 1x concentration, a 10x concentration, and a 100x concentration. The starting concentration for stevia was 0.00026g/500ml. The starting concentration for sugar was 0.02476g/500ml.

Organisms were diluted until countable colonies were achieved. A 115 NTU concentration of organisms was created using a turbidimeter to yield appropriate concentrations of inoculum. Then 1 uL of inoculum was pipetted into each of 14 tubes: two plain broth (control), six stevia, and six

sugar. These tubes were inoculated and incubated overnight at 37°C. Following incubation, 1 uL of each broth was added to 999 uL of saline. This was referred to as dilution one. From dilution one, 100 uL was then added into 900 uL of saline; this was called dilution two and one sample was plated from it, using a 1 uL calibrated loop and a urine culture technique, onto an SBA. From dilution two, 10 uL were then added to 990 uL of saline; two samples were plated from this, using a 10 uL calibrated loop and a 1 uL calibrated loop. Each dilution was plated in duplicate for a total of 42 plates per organism. These plates were then incubated for 24 hours. *Lactobacillus acidophilus* and *B. fragilis* were grown for an additional 24 hours and counted a second time due to small colony size. The colony counts were statistically analyzed using a one sided *t*-test (alpha = 0.05).

Results

The data shows that for *E. coli* and *B. fragilis* there is random variation in growth between sugar and stevia (Figure 1 and 3, respectively). Looking at the chart for L. acidophilus it initially appears that there is an inverse relationship between both sweeteners and the growth of the organism (Figure 2). *t*-tests, which were run using an alpha level of 0.05, consistently demonstrated t-Stat values lower than our t-Critical values (Table 1). These results demonstrate that there are no significant differences in the growth of these organisms between stevia and sugar.

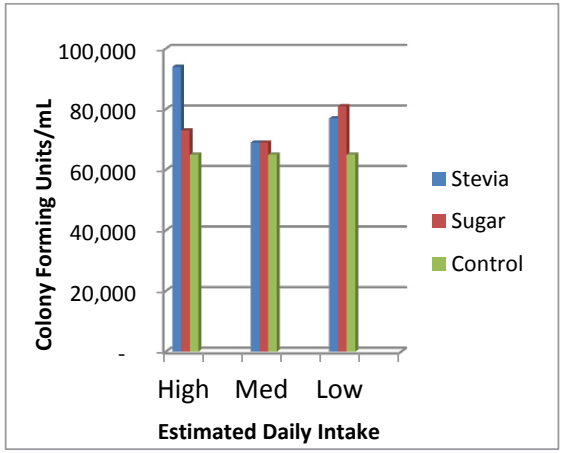

Figure 1. Effect on Growth of Escherichia coli at low, medium, and high concentrations of stevia.

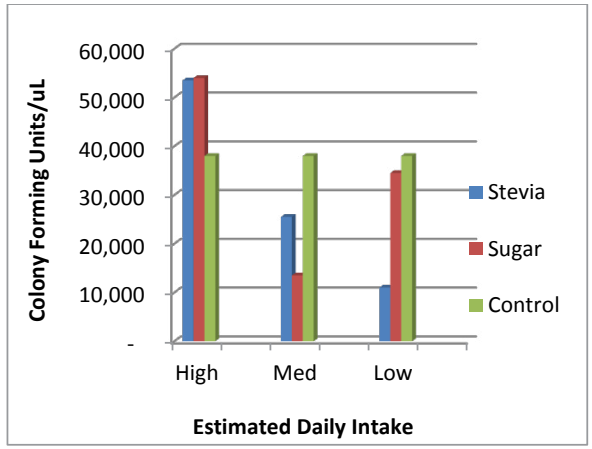

Figure 2. Effect on Growth of Lactobacillus acidophilus at low, medium, and high concentrations of stevia.

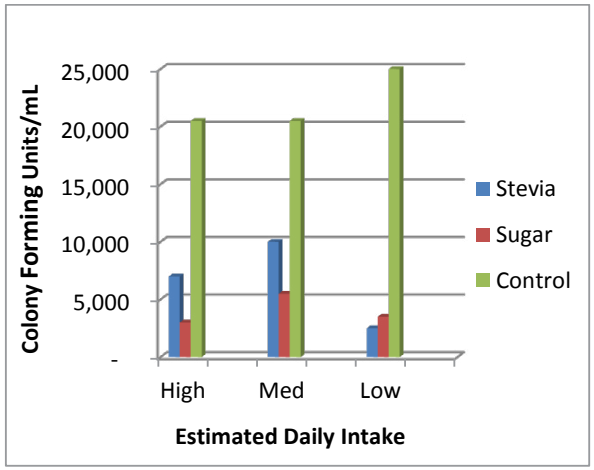

Figure 3. Effect on Growth of Bacterioides fragilis at low, medium, and high concentrations of stevia.

Table 1.

Organism	Vs.	t-Critical	t-Test
E. Coli	Sugar vs. Control	1.2393	2.0613
	Stevia vs. Control	1.2843	2.1788
	Sugar vs. Stevia	0.1016	2.1199
L. acidophilus	Sugar vs. Control	1.0874	2.5706
	Stevia vs. Control	0.8441	2.5706
	Sugar vs. Stevia	1.4019	2.0322
B. fragilis	Sugar vs. Control	0.1384	2.3060
	Stevia vs. Control	0.1384	2.0360
	Sugar vs. Stevia	0.1708	2.0322

Discussion and Conclusion

This study showed no significant differences between the organisms grown in sugar and those grown in stevia (Table 1). As the results of the study conducted on Splenda™ showed a marked decrease in the growth of these organisms, individuals concerned about digestive health may feel more comfortable using stevia (Abou-Donia et al., 2008). Probiotics including *L. acidophilus* and *B. bifidum* have been shown to play an important role in preventing enteric pathogens and parasites from developing (Kailasapathy & Chin, 2000). This is encouraging news for those individuals who have a medical need to limit their sugar intake. Stevia is currently available in a variety of products. Stevia is also available in a granulated form that can be used in baking, so it is feasible for an individual to completely replace their sugar intake with stevia. As always, further studies are needed. The next logical step would be to test this in a living system to determine if the process of metabolizing stevia changes the effects we witnessed. Another study that would be informative would focus on the effect of regular granulated sugar on concentrations of *L. acidophilus* in the body.

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Substance Use, Depression, and Stress: A Retrospective Analysis of their Relations and Trajectories Across Adolescence

Author: Amy Trevethan Blunck
Mentors: Eric Amsel & Leigh Shaw

Abstract: The relationship between adolescent substance use, depressive symptoms, and stress was examined. Ninety-seven participants retrospectively assessed their stress, depressive symptoms, and substance use across their adolescence. Preliminary analysis found similar linear and non-linear trends, highlighting parallel increases in stress, depressive symptoms, and substance use across adolescence. followup analysis found the trajectories of stress and depressive symptom levels were each affected by substantial increases in each other but none affect or were affected by substance use levels. Results point to direct contingencies between trajectories of stress and depression, but not for substance use, in adolescents' retrospective assessments of symptom levels.

Introduction

There has been growing attention to the relation between developmental trajectories of substance use and affective disorders, primarily depression, during adolescence (Kaminer, Connor, & Curry, 2007). Both alcohol dependence and drug abuse are risk factors for depression (Galaif, Sussman, Newcomb, Locke as cited in Siholva, 2008) and it has been shown that an early age of onset of depression strongly indicates later substance abuse (Siholva, 2008). Because of these risks it is important to understand what factors may bring about substance use and depression.

In examining substance use and depression, some researchers have considered their trajectories as altered by a confounded, third variable (e.g., Degenhardt et al., 2003), rather than examining levels of substance use altering levels of depression or vice versa; this Third Variable hypothesis is the approach taken in the present study by examining the levels of stress along with substance use and depression.

The present study was designed to extend substance use and depression research in three important ways. First, the study used a retrospective assessment wherein participants recounted their levels of substance use, depressive symptoms, and stress to allow analysis of their separate developmental trajectories across adolescence. These data, addressed in preliminary analysis, allow for an evaluation of the unique trajectories of symptom changes in a "normal" adolescent sample. Previous research has used either a non-normative adolescent sample (Bukstein, Glancy, & Kaminer 1992), or an adult sample (Bolton, Robinson, & Sareen 2009). Second, the preliminary analysis will provide a qualitative assessment of the Third Variable hypothesis by comparing developmental trends of stress compared to the trend of depressive symptoms and substance use. Third, a followup analysis on participants' time period of substance increases in stress, depressive symptoms, and substance use will examine whether one variable is directly associated with an abrupt change in the others, which will provide direct test of the Third Variable hypothesis.

Methods

Participants

The participants were WSU students between 18 - 22 years of age, recruited from Introductory Psychology classes. The 97 participants (53.6% female, M age = 19.57 years, 73.9% freshmen) received two research credits (a requirement of the Introductory Psychology course) for completing the questionnaire. The sample is largely representative of Introductory Psychology students.

Procedure

All participants completed the questionnaire in addition to a consent, form either in class or at home. The questionnaire contained three types of information: stress symptoms, depressive symptoms, and type of substance used.

Task Items

Participants rated the intensity with which stress symptoms were felt in each year of their adolescence (ages 13 – 22) on a 10-point scale ranging from *No symptoms* to *Highly affecting life*. If participants reported a stress score of higher than 5 for one or more years in their adolescence, they selected the events which contributed to the stress; which these included: *conflict with one or both parents, conflict with peers, academic difficulties, and hormonal/ puberty changes*.

Depressive symptoms were defined by statements used in the National Depression Day Screening Tool and were defined for participants before they completed the depression symptom section. Symptoms included *felt low in energy; had poor appetite; been feeling hopeless about the future; been feeling no interest in things; thought about or wanted to commit suicide*. Following the symptom list, participants indicated at each age (13 – 22) their level of depressive symptoms

from a 5-point scale ranging from *None of the time* to *ll of the time* for each year of their adolescence.

Participants reported their usage of three substances (alcohol, marijuana, and prescription drug abuse) based on how many occasions during each age they used the substance (e.g., alcohol, marijuana, or prescription drugs) on a six point scale ranging from *0* to *20 or more*. Each substance use assessment was completed on a separate chart. Prescription drug abuse was defined as *taken at a higher dosage than prescribed or taken when not prescribed*.

Demographic questions addressed participants' age, sex, marital status (*married, divorced, single,* or *widowed*), and year in school.

Results

Preliminary Analysis

The preliminary analysis was designed to assess the separate trajectories of symptom changes over adolescence. A 6 (Ages: 13 – 18 years old) by 2 (Sex) repeated-measures ANOVA was run for ratings of stress symptoms, depressive symptoms, and substance use (calculated by combining the means of alcohol, marijuana, and prescription drug abuse) to examine the trajectories of each variable separately over time. In addition to main and interaction effects, the analysis provides information about the significance of linear and nonlinear (quadratic, cubic, fourth and fifth order) age effects[1].

Stress

There was a main effect of Age, (F(5,475) = 51.46, p< .001) and Sex (F(1,95)=3.88, p = .052). Female stress ratings (M = 4.80) were higher than those of male (M = 4.06). There was a significant linear, (F(1,95) = 97.98, p< .001), and cubic, (F(1,95) = 12.52, p< .001) trends in Age, reflecting a general rise in stress symptoms as a person ages, with two large increases on the 10-point scale between ages 15 and 16 (M = 1.02) and between ages 16 and 17 (M = 0.81).

Depressive Symptoms

There was a main effect of Age, (F(5,470) = 16.69, p< .001) and a marginal main effect for Sex (F(1,94) = 167.12, p< .01). Females (M = 1.43) had more depressive symptoms than males (M = 0.92). Significant linear, (F(1,94) = 35.90, p< .001), cubic, (F(1,94) = 9.38, p< .01), and 5th order[1] (F(1,94) = 4.69, p< .05) trends were found for Age. These trends reflect an overall rise in depressive symptoms, with two large increases on the 5-point scale found between ages 14 and 15 (M = 0.29 and 16 and 17 (M = 0.29), with additional increases between ages 13 and 14 years and a decrease between 17 and 18 years.

Substance Use

For substance use there was a main effect of Age, (F(5,465) = 14.98, p< .001). There was a significant linear, (F(1,93) = 21.42, p< .001) and cubic (F(1,93) = 4.58, p< .05) trend reflecting a rise in substance use instances over age with two large increases on the 6-point scale between ages 15 and 16 (M = 0.13) and 16 and 17 (M = 0.19).

Followup Analysis

Three analyses employed subsamples of participants for whom a substantial increase in substance use (n = 26), stress (n = 49), and depressive symptom (n = 39) levels were identified in a particular year. A substantial increase was defined as one that was substantially larger for one year compared to the others; this year of substantial increase was defined as year 0 for analysis. For participants with an increase in one symptom (e.g., stress), a separate Year (6) by Sex (2) ANOVA was computed on each of the other two symptoms (e.g., substance use and depression) for the three years previous (-3, -2, -1) and two years subsequent (+1, +2) to the year (0) of the increase. Subsamples were employed because not every participant reported depressive symptoms or substance use and therefore was unable to be examined in this analysis.

Relation of Stress to Other Variables

There were significant linear (F(1,47) = 19.10, p< .001), quadratic (F(1,47) = 8.63, p<.01) and cubic (F(1,47) = 15.39, p< .001) trends when examining stress and depressive symptoms. A significant linear trend, (F(1, 46) = 17.15, p< .001), was found when examining stress and substance use suggesting stress did not alter the trajectory of substance use.

Relation of Depressive Symptoms to Other Variables

Examination of depressive symptoms upon stress trajectory revealed significant linear (F(1,37) = 40.61, p< .001), and cubic (F(1,37) = 13.95, p< .001) trends. A significant linear (F(1,37) = 19.40, p< .001) trend was revealed when examining depressive symptoms and substance use suggesting depressive symptoms did not alter the trajectory of substance use.

Relation of Substance Use to Other Variables

Significant linear (F(1,24) = 12.41, p< .01), quadratic (F(1,24) = 7.40, p< .05), and order 4 (F(1,24) = 4.76, p< .05) trends were found when examining substance use upon stress. The substance use upon depressive symptoms analysis revealed significant linear (F(1,24) = 5.73, p< .05), and quadratic (F(1,24) = 7.34, p< .05) trends.

Discussion

The data analyzed in the present study was designed to assess developmental trends of participants' substance use, depressive symptoms, and stress, as well as examine the impact of one variable's substantial increase on another's trajectory across adolescence. Moreover, the study analyzed the developmental trajectories of substance use and depressive symptoms to suggest evidence of the impact and relation of stress upon these variables.

The similar linear and non-linear trends found in the preliminary analyses point to the inter-relatedness of stress upon substance use and depressive symptoms over adolescence, providing a qualitative test that supports the Third Variable hypothesis.

The prediction from the Third Variable hypothesis that substantial increases in stress would be related to non-linear changes in substance use and depressive symptoms was partially confirmed. Results in the followup analysis show that substance use levels were not directly connected to the year of substantial increases in either stress or depressive symptoms. In contrast, the analysis of stress and depression yielded the predicted non-linear age trend in one symptom level associated with the increase in the other.

The low amount of substances reported warrants an explanation and may be due to two reasons: social desirability bias and the religious affiliation of participants dominating the sample area. This religious group traditionally abstains from substance use. However, it should be noted that significant positive inter-correlation between overall ratings of substance use, depressive symptoms, and stress were found despite the low number of substances reported. This provides an indication of the inter-relation of substance use upon these variables.

The present study has shown that when examined separately, stress shares the same trajectories as substance use and depressive symptoms, consistent with the Third Variable hypothesis. However, when examined directly, only the trajectories of stress and depressive symptoms are contingent upon one another, unlike substance use, in adolescents' retrospective assessments of symptom levels. The findings suggest that future investigations of the impact of stressful events on adolescence is integral to understanding substance use and affective disorders.

Footnote

[1] Non-linear trends reflect a number of different ways symptomology may change over adolescence. A linear trend is reflects an incremental change in which year over year, there is a progressive increase (or decrease) symptoms. A non-linear trend reflect one of many ways of saying that there are changes but that the are not incremental.

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Religiosity Impacting Stepfamily Dynamics as Mediated by Dyadic Adjustment

Authors: Michelle Burton & Andrew Chris
Mentor: Paul Schvaneveldt

Abstract: Stepfamilies face unique challenges as two separate family units attempt to merge into a fully functioning unit (Baxter, Braithwaite, & Nicholson, 1999). The successful transition of two family units into a functioning stepfamily unit requires a strong, unified relationship between the parents of the two family systems (Visher & Visher, 1990). Religiosity is a factor that has been related to marital satisfaction and relationship stability (Ellison, Burdette, & Wilcox, 2010). Research has observed that higher level of marital quality and family cohesion in nuclear families has been found to be related to religiosity (Day, Jones-Sanpei, Smith, Orthner & Hair, 2009).Prest, Russel, and D'Souza (1999) have defined religiosity as a search for meaning, beliefs, and purpose in life regardless of whether it involves a concept of God or not. . Religiosity has further defined as being either intrinsic or extrinsic. Allport and Ross (1967) defined intrinsic religiosity as the internalizing and living out of beliefs and extrinsic religiosity as using these beliefs as a means for social gain

Introduction

While research on stepfamilies has established marital satisfaction as essential to stepfamily success (Visher & Visher, 1990) and religiosity has been shown to increase marital quality in nuclear families (Day, et al., 2009), minimal research has been conducted to establish this relationship amongst religiosity in a stepfamily environment (Lyster, Russell, & Hiebert, 1995). With religiosity having a positive impact on marital relationships in nuclear family systems (Ellison, Burdette, & Wilcox, 2010), there is a need to further study the role that religiosity has in a stepfamily marital dyad. The current study examines the impact of religiosity on the marital dyad of a stepfamily and in turn if there is a mediating impact on stepfamily dynamics from the marital dyad.

Stepfamily Dynamics

Stepfamilies face a myriad of obstacles as they attempt to merge two separate families into one healthy unit. In order for a healthy level of cohesion to be obtained, relationship dynamics within the family must be

a stepfamily environment can dictate the growth of solidarity within the family unit (Baxter L. A., Braithwaite, Bryant, & Wagner, 2004). Positive communication amongst the marital dyad has been related to healthier family functioning, higher levels of cohesion, and lower levels of boundary ambiguity (Bello, Brandau-Brown, & Ragsdale, 2008). In contrast, Schrodt (2008) observed that lack of positive communication patterns creates more uncertainty of roles within a stepfamily, of particular concern with stepparent/child interactions. The style of communication a stepfamily environment interacts with is a predicting variable in developing solidarity within the family unit (Visher & Visher, 1990).

Religiosity and Couple Dynamics

Religiosity is a variable that remarried couples identify as being important to their family functioning as well as their own relationship (Lyster, Russell, & Hiebert, 1995). Research has indicated that marital satisfaction can be enhanced through shared interactions and beliefs in the spiritual sense (Call & Heaton, 1997). Further studies have indicated higher rates of commitment amongst marital dyads when engaging in religious activities (Marks, 2006). Marital partners who engage in mutual religious activities are less inclined to hold spiritual-based beliefs against their partners and more willing to work together to reach solutions to problems (Rootes, Jankowski, & Sandage, 2010).

Religiosity has been found to be a factor in impacting negative conflict interactions within marital dyads (Lambert & Dollahite, 2006). Jose and Alfons' (2007) research indicated that marital dyads with shared spiritual beliefs engaged in higher rates of forgiveness behavior when experiencing conflict. Higher levels of commitment for both partners to their principles (Marks, 2006) and beliefs that transcend the couple (Greenway, Phelan, Turnbull, & Milne, 2007) have been observed to be healthy coping strategies for marital dyads in conflict. Further research has indicated couples with high religiosity exhibit more positive communication interactions as they engage in problem solving strategies around conflicts dealing with parenting, finances, and relationship roles (Call & Heaton, 1997; Rootes, Jankowski, & Sandage, 2010). Research has indicated that families who experience high levels of conflict and dissolution have lower rates of religiosity (Day, et al., 2009).

Couple Dynamics and Family Process

For stepfamilies to reach a sense of solidarity, a strong marital dyad is required as the foundation for a cohesive family unit to develop (Visher & Visher, 1990). Cissna, Cox, and Bochner's (1990) research indicates that couples more open in their communication are also clearer in terms of their

parenting, setting expectations for their children, and rules for disciplining. In contrast, Golish (2003) has observed that negative communication interactions, such as criticizing a previous spouse or stepchild, leads to a lack of family unity and higher rates of family discord. Studies have also indicated that a healthy marital unit provides a positive example for the children of the two merging families (Baxter, Braithwaite, & Nicholson, 1999). As children experience a healthier marital relationship, they feel a higher sense of belonging and willingness to work together as a unit (Leake, 2007).

Hypothesized Model

Research has been conducted on couple dynamics influencing family process as well as religiosity's impact on couple dynamics. However, no previous research has studied a model testing these variables simultaneously. Religiosity's impact on stepfamily dyadic adjustment has also been studied minimally. With research indicating relationships between religiosity and marital dyadic adjustment in nuclear families (Call & Heaton, 1997), this study seeks to observe this same relationship in a stepfamily environment. Further research has specified marital dyadic adjustment influencing the dynamics of the family unit (Visher & Visher, 1990). The current study aims to combine the variables of religiosity and dyadic adjustment to observe the impacts these variables have on stepfamily dynamics.

The full-hypothesized model posits that intrinsic and extrinsic religiosity will be related to the dyadic adjustment of the stepfamily marital couple. The model posits that the dyadic adjustment of the couple will be related to Step family dynamics on three domains: Family Problem Solving, Negative Conflict Interactions, and Family Self-Disclosure. The model additionally posits that intrinsic and extrinsic religiosity will be related to the three-stepfamily domains of: Family Problem Solving, Negative Conflict Interactions, and Family Self-Disclosure.

Methods

Subjects

A total of 307 subjects constituted the national sample of participants involved in this study. The sample consisted of 191 males (62.2%) and 115 females (37.6%), with one participant not denoting their sex. The mean age of participants in this study was 39.36 (SD= 7.88. Subjects were recruited through their affiliations with the National Stepfamily Day organization and the Blended and Stepfamily Resource Center. Participants associated with these national associations were able to voluntarily access the survey through

an online link. A $5 e-certificate incentive was available for participants if they chose to be compensated with confidentiality of their responses guaranteed. Criterion for subject participation included a current marriage partner in which the marriage produced a stepfamily environment.

Materials

Levels of participants' intrinsic and extrinsic religiosity were measured through the use of Allport's Religious Orientation Scale (ROS) (Allport & Ross, 1967). The ROS has a measured Cronbach's alpha reliability estimate of .84 with regards to measuring intrinsic religiosity and .78 for extrinsic religiosity, representing a high level of internal consistency. Communication within the stepfamily marital dyad was measured with the use of the Primary Communication Inventory (PCI) developed by Locke, Sabaght, and Thomes (Corocan & Fisher, 2000). The PCI has been shown to have excellent levels of concurrent validity. Current levels of reliability are unknown, with excellent levels of concurrent validity being assessed. Cohesion levels associated with the stepfamily environment were assessed via the Index of Family Relations (IFR), a scale developed by Hudson (1997). The IFR has been found to have a Cronbach's alpha reliability estimate of .95. This represents a high level of internal consistency for this survey. The quality of the marital dyad was measured with the Dyadic Adjustment Scale (DAS) developed by Spanier (1976). The Dyadic Satisfaction of the DAS was used to measure conflict and has a Cronbach's alpha level of .94. This represents a high level of internal consistency.

Procedure

The Religious Orientation Scale (ROS), Primary Communication Inventory (PCI), Index of Family Relations (IFR), and Dyadic Satisfaction subscale were compiled in the form of an online survey. Participants were able to access the survey by opening the link provided by the National Stepfamily Day organization and the Blended and Stepfamily Resource Center on their websites. Upon completion of the survey, participants were able to input their email address to receive a $5 e-certificate incentive.

Data Analysis

Before completion of the structural equation model, a sum score for participant's answers was calculated. A structural equation model was tested to identify the impacts of intrinsic and extrinsic religiosity on dyadic relations and stepfamily relational dynamics. Factor analyses were conducted using a maximum likelihood method with direct oblimin rotation to identify potential latent variables. Latent constructs were identified using a

confirmatory factor analyses and were included in the full-hypothesized models.

Results

Sample

The current study employed a national sample of 307 participants consisting of 191 (62.2%) males and 115 (37.6%) females, with one participant omission (see Table 1). The mean age of participants was 39.36 (SD=7.88). Further demographic information is included in Table 1.

Table 1. Demographic Characteristics of the sample

Male	191	62.2%
Female	115	37.6%
Age	39.36	SD 7.88
Level of Education		
High School Degree	5	1.6%
Vocational/Technical School	11	3.6%
Some College	67	21.8%
Bachelor's Degree	131	42.7%
Some Graduate School	33	10.7%
Master's Degree	57	18.6%
Doctoral Degree	3	1%
Religious Affiliation		
Christian	106	34.5%
Catholic	58	18.9%
Mormon	28	9.1%
Protestant	23	7.5%
Atheism	7	2.3%
Other	85	27.7%

Measurement Models

Two exogenous constructs and two endogenous constructs that comprise the full hypothesized structural model are shown in Figures 1 and 2.

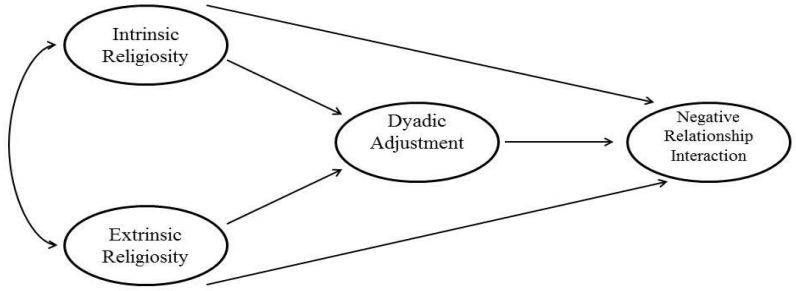

Figure 1. Hypothesized Structeral Model of Negative Relationship Interactions

Intrinsic religiosity was defined by five items including such items as religion keeps my life balanced or being aware of the presence of God or the Divine Being (see Table 2). The extrinsic construct was defined by six items including church is important for good social relationships or congenial social activity. Dyadic adjustment was defined by eleven items including agreeing on matters of recreation or religious matters. Goodness of fit with standardized factor loadings of .5 was found for all latent constructs.

Negative relationship interactions, an endogenous construct, were defined by seven items that included items such as sense of closeness in family or family arguing too much. Family communication was defined by six items with examples including frequency of communication about items of disagreement or knowing what your spouse is trying to say. Goodness of fit with standardized factor loadings greater than .5 were found for this endogenous construct (see Table 2).

Table 2.

Standardized Factor Loadings of Confirmatory Factor Analyses of Latent Constructs

	Standardized Factor Loading
Intrinsic Religiosity	
Religion helps keep my life balanced.	.44
Quite often I have been keenly aware of the presence of a Divine Being.	.50
My religious beliefs are behind my whole approach to life.	.45
I try to carry my religion into all my other dealings in life.	.49
It is important to spend time in private religious thought.	.57
If not prevented by unavoidable circumstances, I attend church.	.58
Religion is important because it answers questions about the meaning of life.	.68
I read literature about my faith (or church).	.64
Extrinsic Religiosity	
Church is an important place to formulate good social relationships.	.46
A primary reason for religion is congenial social activity.	.57
I refuse to let religious considerations influence my everyday affairs	.52
I feel there are many more important things in life than religion.	.54
I pray chiefly because I have been taught to pray.	.59
Being a church member helps to establish a person in the community.	.67
Sometimes it is necessary to compromise religious beliefs to protect my social and economic well-being.	.68
Family Communication	
Frequency that your spouse discusses matters of sex with you.	.46
Feels that in most matters your spouse knows what you are trying to say .	.43
Frequency that you and your spouse talk about personal problems.	.55
Frequency that you and your spouse talked most things over together.	.57
You and your spouse discuss things together before making important decisions.	.49
Frequency that you and your spouse talk over unpleasant things from the day.	.59
Negative Family Interactions	
There is no sense of closeness in my family	.75
Members of my family argue too much.	.67
I feel like a stranger in my family.	.83
I wish I was not part of this family.	.72
My family does not understand me.	.74
There is too much hatred in my family.	.69
I really so not care to be around my family.	.58
Dyadic Adjustment	
My partner and I agree on handling family finances.	.53
My partner and I agree on religious matters.	.55
My partner and I agree on conventionality.	.59
My partner and I agree on ways of dealing with in-laws.	.58
My partner and I agree on aims, goals, and things believed important.	.70
My partner and I agree on amount of time spent together.	.67
My partner and I agree on making major decisions.	.56
My partner and I agree on friends.	.66
My partner and I agree on sex relations.	.66
My partner and I agree on leisure time interests.	.65
My partner and I agree on career decisions.	.73

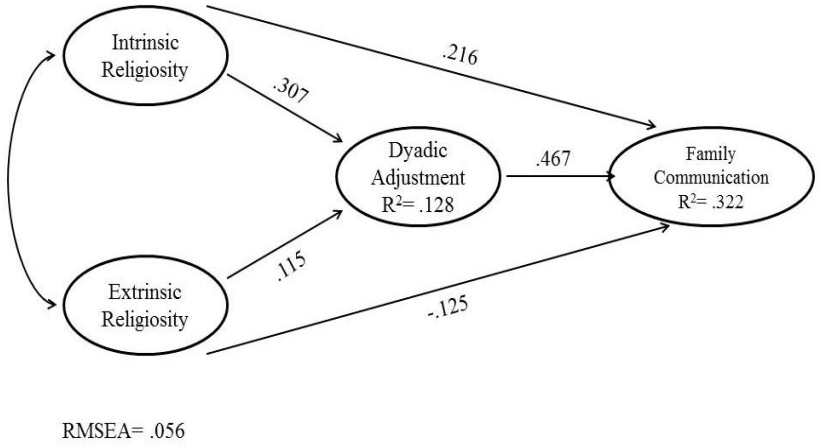

Figure 3. Revised Full Hypothesized Structural of Family Communication

Several paths in the model were statistically significant at a probability level of .01 or better. Extrinsic religiosity was found to positively predict more negative relationship interactions within the family (.413), whereas intrinsic religiosity only weakly predicted negative relationship interactions (.037). Dyadic adjustment was found to negatively predict negative relationship interactions (-.407).

Religiosity

The relationship between religiosity and negative relationship interactions revealed a dichotomy between intrinsic and extrinsic religiosity. Intrinsic religiosity was found to positively predict dyadic adjustment (.306) and only weakly predict negative relationship interactions (.037). Extrinsic religiosity was found to positively predict dyadic adjustment (.111) and negative relationship interactions (.413). These results indicate that higher levels of extrinsic religiosity predict higher levels of negative relationship interactions amongst the marital dyad.

Dyadic Adjustment

Dyadic adjustment was found to negatively predict the levels of negative relationship interactions (-.407). These results indicate the higher levels of dyadic satisfaction the lower levels of conflict, or vice versa.

Results of the full-hypothesized structural model for Family Communication showed a good fit to the data with a RMSEA= .056. The model explained 32% of the variance of Family Communication (see Figure 4).

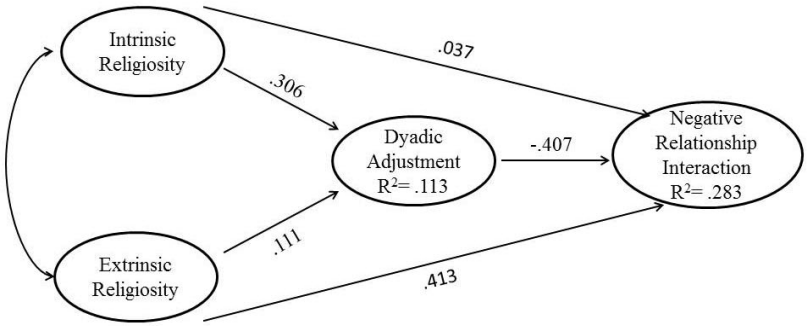

Figure 4. Revised Full Hypothesized Structural Model of Negative Relationship Interaction

Several paths in the model were statistically significant at a probability level of .01 or better. Intrinsic religiosity was found to positively predict more family communication (.216), whereas extrinsic religiosity was found to negatively predict family communication (-.125). Dyadic adjustment was found to positively predict family communication (.467).

Religiosity

The relationship between religiosity and family communication again revealed the dichotomy between intrinsic and extrinsic religiosity. Intrinsic religiosity was found to positively predict dyadic adjustment (.307) as well as family communication (.216). Extrinsic religiosity positively predicted dyadic adjustment (.115) and negatively predicted family communication (-.125). These results indicate that higher levels of intrinsic religiosity not only predict higher levels of dyadic adjustment, it also positively influences family communication. Extrinsic religiosity in contrast positively predicts dyadic adjustment, however negatively predicts family communication.

Dyadic Adjustment

Dyadic adjustment was found to positively predict family communication (.467). These results indicate that higher levels of dyadic satisfaction increase the likelihood of family communication.

Discussion

The current study investigated the impact religiosity has on dyadic adjustment and stepfamily process. Currently there is limited research assessing religiosity's impact on family process in a stepfamily environment as well as dyadic adjustments mediating impact on family processes. Research that has been conducted on this topic has focused on nuclear families, presenting the need for exploration into the stepfamily environment. Studies conducted with nuclear families have provided indications of religiosity and dyadic adjustments positive impact on family process. From this research the full-hypothesized structural model was developed.

Results show support for the full-hypothesized structural model in that intrinsic and extrinsic religiosity explained step-family dynamics as moderated by dyadic couple satisfaction. The model posited that dyadic adjustment would be influenced by the type of religiosity, intrinsic or extrinsic, and in turn would mediate the stepfamily process, negative relationship interactions or family communication.

In regards to the family process of negative relationship interactions, dyadic adjustment was found to negatively predict this process. Higher levels of dyadic adjustment resulted in lower levels of negative relationship interactions; the reverse is also supported. Dyadic adjustment for this model was found to be impacted by the type of religiosity, with both intrinsic and extrinsic religiosity impacting dyadic adjustment. Regardless of the type of religiosity, higher levels of dyadic adjustment was predicted, however intrinsic religiosity accounted for higher levels of dyadic adjustment. Religiosity was also found to predict the amount of negative relationship interactions. Extrinsic religiosity was found to positively predict higher amounts of negative relationship interactions seemingly indicating higher levels of extrinsic religiosity in a stepfamily setting fosters an environment of more negative relationship interactions.

In regards to the family process of family communication interactions, dyadic adjustment was found to positively predict this process. Higher levels of family communication were found to be present when dyadic adjustment was high. This result indicates the relationship of the couple has a positive influence on the communication of the family. Dyadic adjustment for this model was impacted positively by religiosity, indicating higher levels of religiosity predict higher levels of dyadic adjustment. In terms of type of religiosity, intrinsic religiosity had a higher positive influence on dyadic adjustment. In terms of religiosity and family process, it was found that intrinsic religiosity positively predicted family communication. The more intrinsic the environments of the stepfamily, the higher levels of family communication present. In contrast, an environment of extrinsic religiosity

negatively predicted the amount of family communication. Family communication came in lower amounts when families adopt more of an extrinsic attitude towards religiosity.

Religiosity's positive impact on dyadic adjustment in this study is supported by Call and Heaton (1997) whose research has found a connection between marital satisfaction and religiosity. The models presented in this research further fit the current data when assessing dyadic adjustments influence on family process. Golish (2007) found that the marital relationship is a primary factor in influencing family cohesion and communication. The current model supported these findings, indicating that higher levels of dyadic adjustment led to more positive family communication patterns and less amounts of negative relationship interaction.

Implications

The current study's results lead to several implications for family life education and therapeutic settings. Findings of the model add to the body of knowledge that the couple relationship is significant in predicting levels of family conflict in a stepfamily environment. With an understanding from the research, professionals can aid stepfamily environments by increasing the awareness that the dyadic adjustment is crucial in stepfamily functioning. While the spousal relationship is not the only variable used in predicting stepfamily success, it is a dynamic that has consistently been found in the research to support healthy stepfamily functioning.

The results of the current study also suggest that religiosity may be beneficial in promoting healthy couple relationships. Results of the model also indicate the type of religiosity has an impact on healthy couple relationships and family functioning as well. Intrinsic religiosity was found to be positively related to dyadic adjustment, family communication, and lower levels of negative relationship interactions. Family life educators and therapists can use this finding to assist stepfamilies through emphasizing the need to truly believe and instill the values of common beliefs together. Stepfamilies that share a common belief as well as living this belief in an intrinsic way have higher levels of family communication and less negative relationship interactions.

Limitations

The current study is hindered in that a longitudinal design was not used to assess religiosity's long-term impact on family functioning. A longitudinal design would allow for family processes to be studied at multiple intervals, giving a better indication of the long-term impact these variables have in assisting family functioning. Further, while the sample is a national sample, it does not represent the true population nor was it a true random sample.

To read more contact michelleburton@hotmail.com for a copy of the complete study.

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Psychology

Personality, Religiosity, and Religious Denomination

Author: Michael Brown
Mentor: Aaron Ashley

Abstract: Many striking patterns of correlation were found when religious denominations were discriminated. In particular, the LDS portion of our sample was found to have many significant correlations between religiosity and personality variables. Many of these significant findings were conspicuously absent among the other denominational groupings. Patterns of correlations were found to be contingent upon stated religious denomination.

Introduction

The interest of religion in the field of psychology has been well established. The origins of this interest can be traced as far back as William James (1902) who argued that religion had both positive and negative aspects. Sir Francis Galton empirically studied the efficacy of prayer even before psychology was established as a science (Galton, 1872). Within the context of his Oedipal Theory, Freud viewed god as an imaginary father figure whom human beings created in order to deal with death anxiety and as a perceived source of protection and support. According to Freud, "Religion is an illusion and it derives its strength from the fact that it falls in with our instinctual desires." Because of his all knowing and ubiquitous nature god was also considered to be a consistent source of fear and guilt. Because of these perceived negative aspects of religion Freud was very hostile towards it (Freud, 1933).

Religiosity

In the varieties of religious experience, William James described two kinds of religious individuals who fall at different ends of a continuum. He placed the "sick souls" at the low end of the continuum. He explained that these are the kinds of people who constantly dwell on the negative aspects of existence and human kind and define their existence in terms of suffering yet use religion as a perceived liberation from this suffering. For the "sick souls" this liberation was always projected into the afterlife and thus never experienced during life. On the other extreme of the continuum we find what James considers to be the healthy minded, those who praise and thank god for everything that they have. These are the "healthy minded" people, and thus he concludes that religion can play both positive and negative roles (James, 1902). Various mystical and religious beliefs and practices have been

ubiquitous throughout the history of humankind and have always been a topic of interest. The scientific study of religion on the other hand is a relatively recent scientific inquiry. Further expanding on the religious constructs of Freud, Ellis subsequently lumped religion into the same category as his famous "irrational beliefs". For example; in Ellis' view religion thus became akin to the mistaken belief that one must be world-class in all respects in order to be a worthwhile person (Ellis, 1965). Among the earliest to create measures of religiosity that could be tested empirically was Allport (1959). Allport distinguished between individuals who tended to practice their religion for instrumental purposes ("extrinsic") in order to gain some other end such as social approval or to gain access to a community to interact with and conversely, those who practiced their religion for reasons such as a personal conviction or a personal sense of satisfaction ("intrinsic"). These two constructs became the basis for the two aspects of religiosity that are commonly analyzed in today's scientific research, extrinsic and intrinsic religiosity. These were further organized into the ROS (Religious Orientation Scale).

A third construct was later added by Batson to help make distinctions beyond the extrinsic-intrinsic paradigm. This new aspect of Religiosity was called Quest, "the degree to which an individual's religion involves an open-ended, responsive dialogue with existential questions raised by the contradictions and tragedies of life" (Batson, 1976). This view of religiosity represents those who are open-minded in their religious practice, search for answers, and arguably could even be described somewhat agnostic. A variety of methods have been used in attempt to measure religiosity, but the most recent measure of this construct is the Revised Religious Life Inventory, (RLI-R), (Hills et al. 2005). These three constructs formed the basis for the RLI (Religious Life Inventory), (Batson & Schoenrade, 1991). Over a decade later the items on this scale were reviewed and factor analyzed to improve reliability, particularly on the Quest items (Hills et al., 2005). Today this is the most accurate and comprehensive method of measuring religiosity.

Personality

Traditionally, personality has been a difficult construct to characterize and measure due to widespread disagreement and multiple scales and conceptualizations that seemed to go on forever (Goldberg, 1971). Therefore there was little consensus among researchers and a lack of validity across different personality measures because scales that would appear to measure the same thing actually turned out to be measures of different constructs, and conversely, measures that appeared to measure different things turned out to be measures of the same construct. This made communication among researchers extremely difficult. Allport wrote of this problem and its

implications rather concisely, "Each assessor has his own pet units and uses a pet units and uses a pet battery of diagnostic devices" (Allport, 1958).

Current conceptualizations of personality typically employ the Five-Factor Theory (Costa & McCrae, 1988), which is a result of decades of research and factor analysis. It provides a descriptive account of five core personality characteristics. These characteristics are commonly known by the acronym OCEAN.

The profile of someone who scores high on Openness to Experience is one who is creative, prefers variety, and has a wide array of interests. Liberal values are commonly associated with this factor (Costa & McCrae, 1992). Conscientiousness is the extent to which someone is dependable, controls their impulses, and works hard towards the goals that they set for themselves. Conscientious individuals value cleanliness and ambitiousness. Conscientiousness is widely rewarded in schools and most institutions and this is thought to contribute to the finding that Conscientious individuals typical have high self-esteems (Costa et al., 1991). Extraversion is what characterizes those who are talkative and passionate. It is the opposite of being shy, and compared to their shy peers they are more likely to report falling in love during college. The extravert values an exciting life and being happy and cheerful. Agreeableness is the factor of personality which characterizes someone who is compliant and friendly. They report little conflict in their interpersonal relationships. A survey of those scoring high on Agreeableness indicated that they tend to value forgiveness, helpfulness, and love.

And finally, Neuroticism describes individuals who are frequent worriers, and are characterized by insecurity. Neuroticism is associated with negative emotions such as anxiety and depression. Neurotics commonly report having been exposed to a wide range of disturbances, which may explain their generally negative pattern of emotional responding.

Religiosity and Personality

Research into the area of religiosity has revealed numerous individual differences, and associations, particularly in terms of personality. One finding that is particularly pervasive is a negative relationship between religiosity and psychoticism (Eysenck, 1998). Other studies have found religion to be associated with the personality trait Agreeableness, since these traits are the opposite of psychoticism this indirectly confirms findings such as Eysenck's. Specific patterns between religion and other FFM (Five-factor model) personality traits have been inconsistent in the literature (Saroglou, 2002).

Another study of 422 music students found that the personality traits extraversion and neuroticism are not related to religiosity as measured in terms of such factors as personal prayer and church attendance. This study confirmed the general finding of a negative correlation between religiosity

and psychoticism, thus adding support to the notion that music students do not score differently from the general population, and adding confidence to the generalizability of our religiosity measurements (Bourke & Francis, 2000). In a sample of Muslim Kuwaiti undergrads it was found that those who were considered religious tended to have lower scores on neuroticism (Abdel-Khalek, 2010). And yet another study found that scores on Religiosity had low correlations with neuroticism (Schwab & Petersen, 1990).

One hypothesis and justification for studying the relation between religiosity and personality is the possibility of being able to predict religiosity on the basis of personality traits. This was investigated in a longitudinal study which revealed that in later adult life religiosity tends to have a positive correlation to Conscientiousness and Agreeableness. Openness to Experience was related to spiritual seeking. Further elucidated by this study was the finding that adolescent personality traits can impact religious tendencies in later life. For example Conscientiousness in adolescence significantly predicted religiosity in later adulthood (Wink, Ciciolla, Dillon, & Tracy, 2007).

A recently published meta-analysis of 71 studies and their samples (N=21,715) which contains subjects from 19 countries as well as a literature review on personality and religion found that individual differences in religiosity can (at least partly) be explained as "a cultural adaptation of two basic personality traits, Agreeableness and Conscientiousness" (Saroglou, 2010). These findings were consistent independent of many factors, such as the dimension of religiosity being studied, which personality measure was used, or context (e.g. gender, age, or country of origin). In general religiosity was not related to Extraversion and Openness to Experience, which suggests that religiosity is not compatible with "key functions of human personality (e.g., plasticity and growth)". Saroglou thus concludes that religiosity represents "a human concern for personal and social stability and moral self-transcendence but not the human needs for playfulness, personal growth, and social change." Essentially this means that religious people and atheists/non-believers have played complementary roles in society in terms of shaping what it has become, with religion contributing to "the establishment" (temporal stability of social constitution), and providing moral examples. Conversely, the competing viewpoints (such as atheism) have provided the "entertainers, creators, rebels, and revolutionaries" (Saroglou, 2010). Henningsgaard & Arnau (2008) reported many significant correlations between the dimensions of the RLI-R and the five factors of personality. This study expands on their findings. It highlights some key similarities and differences between religious denominations.

Methods

Participants

Participants in this study consisted of 258 college students (186 females, 72 males) ranging in age from 16 to 53 (M = 22.02 years, SD = 6.14) who were enrolled at Weber State University in Ogden, Utah. The participants received course credit for completing the questionnaires.

Materials

Each participant completed the three subscales of the RLI-R; "extrinsic" (religion as a means), "intrinsic" (religion as an end), and "quest" (an interactive way of finding meaning), the Big Five Inventory (BFI), (Costa & McCrae, 1988), and a demographic measure. Prior to analysis, the subjects were separated into three groups on the basis of self-reported religious denomination: LDS, Other (any other stated denomination), and None (stated agnosticism or atheism).

Based on pervasive findings it was expected that we would be likely to find the personality traits Agreeableness and Conscientiousness to be related to religiosity, and that neuroticism would be negatively related to religiosity.

Results

Many significant correlational patterns between religiosity and personality variables such as Agreeableness were discovered among the LDS sample. Preliminary analyses also suggest that different patterns of correlations exist between religiosity and personality among our LDS sample when compared to Henningsgaard's (2008) Baptist-majority sample. See Table 1.

As previously mentioned the participation data was seperated solely on the basis of stated denomination. As expected similar religiosity correlations between the "Other" group (any stated religious denomination besides LDS) and the LDS group were found. Yet curiously specific relationships between religiosity and personality factors differ among these two denominational categories. See Table 2.

Different correlations were found among the religiosity variables for those who declared they had no religious affiliation or beliefs (atheism or agnosticism), in particular a strong positive correlation (r = .68) was found between RLI-E and RLI-Q. Although the religiosity patterns are different from the LDS group, their patterns of personality correlations are similar. See Table 3.

Correlation coefficient comparison tests were then conducted for each variable to evaluate group differences. The top half of the diagonal matrix separation in Table 4 shows correlational differences that are statistically significant between the LDS and Other groups. The bottom half of the diagonal matrix separation shows correlational differences that are significant between the LDS and None groups. See Table 4.

Similar correlation patterns exist for each of the dimensions of religiosity in all three groups. However, LDS-Other and LDS-None group comparisons show different correlational patterns between religiosity and personality. No significant differences were found in the correlations between the Other and None samples.

As expected based on previous research, Agreeableness had positive correlations, with all three dimensions of religiosity (RLI-E=.177), (RLI-I=.027), (RLI-Q=.072), but this was only for the non-LDS subjects. Two of the correlations with Agreeableness ran strongly negative in the opposite direction for the LDS subjects (RLI-E=-.164), (RLI-Q=-.154), however on the third aspect of religiosity a much stronger correlation between RLI-I and Agreeableness was found.

Table 1. Correlations between RLI and Big Five (LDS, n=165)

	RLI-I	RLI-Q	O	C	E	A	N
RLI-E	**-.40****	**.36****	-13	**-20***	-.02	**-.16***	-.02
RLI-I		-.07	**.21****	.12	.09	**.24****	-.03
RLI-Q			.09	-.09	-.10	**-.15***	.05
O				.14	**.23****	.11	**-.17***
C					.11	**.25****	**-.17***
E						-.02	**-.26****
A							**-.24****

* = p<.05 ** = p<.01

Note: Significant and contrasting patterns of correlations between religiosity and personality variables such as Agreeableness are present

Table 2. Correlations between RLI and Big Five (Other, n=65)

	RLI-I	RLI-Q	O	C	E	A	N
RLI-E	-.21	**.39****	-.10	-.24	.16	.18	.02
RLI-I		-.01	-.17	.05	-.01	.03	-.10
RLI-Q			-.01	-.06	.10	.07	.09
O				-.03	.14	.21	-.15
C					-.22	**.29***	-.13
E						.16	-.10
A							**-.41****

* = p<.05 ** = p<.01

Note: Similar religiosity patterns exist with the LDS group, yet the relationships between religiosity and personality factors differ

Table 3. Correlations between RLI and Big Five (None, n=25)

	RLI-I	RLI-Q	O	C	E	A	N
RLI-E	.04	**.68****	-.13	-.11	-.05	-.06	.23
RLI-I		-.35	-.17	.16	-.01	.22	-.16
RLI-Q			.06	-.01	-.26	-.05	.11
O				.09	.026	.11	**-.47***
C					.14	.32	.09
E						.08	-.31
A							-.06

* = p<.05 ** = p<.01

Note: The religiosity patterns are different from the LDS group, yet their pattern of personality correlations are similar

Table 4. Significant Correlation Coefficient Differences LDS/Other

LDS/Other	RLI-E	RLI-I	RLI-Q	O	C	E	A	N
RLI-E	--						-.34	
RLI-I	-.44	--		.38				
RLI-Q	-.32	-.42	--					
O				--				
C					--	.33		
E						--		
A							--	
N								--

Discussion

Among the LDS sample significant correlations were found with all three measures of religiosity. Interestingly, this pattern was absent for the other groups. The LDS sample and the "Other" sample show a similar pattern of correlations on the religiosity variables, yet the way that these variables interact with different personality traits vary considerably between the groups. The "None" sample had a similar pattern of correlations to the LDS sample on the personality factors, yet not on the religiosity factors. In terms of the religiosity and personality variables noted in Table 4, LDS appears to be qualitatively different from those professing other religions. The strikingly different pattern of correlations between the denominations elucidates our understanding of the religiosity construct. A more complete understanding of religiosity will involve more than observing simple one-to-one relationships among religiosity and personality. Religious denomination is a major factor to be considered in future conceptualizations of religiosity and personality.

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A Comparative Study of Delinquent Youth and Parent/ Guardian Perceptions of Risk Factors

Authors: Todd Spencer, Alex Allen, Nancy Chris, Merilyn Smith, Amber Collings
Mentor: Qiu Wei-Arthus

Abstract: There has been a great deal of research looking at the risk factors that contribute to adolescent delinquency, but there has been insufficient research comparing the perceptions of both the youth and their parents of what risk factors led to criminal behavior. To test parent and delinquent agreeableness, 36 parent and youth pairs from Salt Lake Peer Court were given surveys looking at family relationship dynamics, aggressive and depressive emotions, the presence of drug use, the role and influence of peers, school participation, and academic achievement. The results conclude that adolescents and parents have a high level of agreeableness to what risk factors led to risk behaviors.

Literature Review

Delinquent adolescents usually have several risk factors that play a role in their delinquency (Rosewater, 2003). Risk Factors are those characteristics of the person or the environment that are associated with an increased probability of maladaptive developmental outcomes (Compas et al., 1995). Risk behaviors are activities adolescents are participating in that may hinder a healthy development. Most often risk behaviors are closely associated with risk factors (Compas et al., 1995). Parents who know where their child is, who they are with report lower levels of delinquency (Lahey et al., 2008). Family background can also be a risk factor for juvenile delinquency (Rumberger, 2004).

Methodology

The purpose of the project is to examine what risk factors adolescent delinquents attribute their behavior to in comparison to what their parent or legal guardian reports to be the factors that led their teen towards criminal activity.

Research Design

To conduct this project a cross sectional design was used. Our survey asked a variety of questions involving potential risk factors and behaviors

associated with delinquent behavior. This instrument was given to both the adolescent delinquent, aged 12-18, and their accompanied parent or legal guardian. This allowed us to observe common responses as well as discrepancies in the response of what factors are involved.

Sampling

Participants in the study were a purposive sample of 36 parents and 36 youth participating in the Salt Lake Peer Court system that serves youth involved in a delinquent act. Of the 36 parent/guardian surveys, 23 (63.9%) were completed by females and 11 (30.6%) were completed by males with two unlabeled. The average age of the parent/guardian was 37.36 years and the average number dwelling in their household reported was 4.77 members. Of the 36 parent/guardians participating, 5 were Caucasian (13.9%), 26 were Hispanic (72.2%), 2 were Native American (5.6%), 2 were Bi-racial (5.6%), and 1was left blank. In addition, 13 (36.1%) were single, 4 (11.1%) were co-habitating, 12 (33.3%) were married, 5 (13.9%) were divorced, 1 (2.8%) was widowed and 1 was left blank. The annual income level reported that 12 (33.3%) were in the $0-$15,000 range, 14 (38.9%) were in the $15,000-$30,000 range, 9 (25.0%) were in the $30,000-$45,000 range and 1 was left blank. Education acquired showed 1 (2.8%) with no schooling, 6 (13.5%) attended grade school only, 11 (30.6%) had some high school, 10 (27.8%) graduated from high school, and 6 (16.7%) had attended some college, with 3 left blank.

Instrument

Both the adolescent and parent survey are based of off existing instruments developed by the National Institute on Drug Abuse (NIDA). For the adolescent survey we selected 39 out of the 139 questions from the Problem Oriented Screening Instrument for Teenagers (POSIT). For the parent/guardians survey 39 out of the 139 questions were selected from Problem Oriented Screening Instrument for Parents (POSIP).

The questions selected for our study were chosen based on what questions best supported our study. Both the POSIP and POSIT were yes/ no questionnaires, we felt that by using a 5-point likert scale from “Strongly Agree” to “Strongly Disagree” we could obtain a more accurate picture of both the adolescent and parent’s perceptions.

Data Collection

Prior to conducting research, permission was obtained from the Salt Lake Peer Court to conduct research on the youth and their parent/guardian for this study. An informed consent letter was given to participating parent/ guardian that required their signature allowing their child to participate in

the survey. The consent letter was translated from English into Spanish three times to insure correct translation.

Data was collected on four consecutive Thursday evenings, March 17, 24, 31, and April 7, at the Scott Matheson Courthouse in Salt Lake City. The researchers would meet with the parents/guardians and adolescent as they waited for their assigned time. A spanish speaking translator was available for those participants who required such assistance.

Data Analyses

Data will be analyzed using the SPSS or Statistical Package for Social Sciences. We have a series of questions that can be categorized into four main areas (community, peers, family, and individual) that we will analyze through this process.

Hypothesis 1

There will be a significant difference between parent/guardian's and adolescent's perceptions of family relationships as a risk factor of juvenile delinquency.

Table 3. Results of Paired Samples t-Test on Perceptions of Family Relationships

Family Relationships	N	Mean	SD	t	df	Sig
Adolescents	25	19.84	6.07	-.495	24	.625
Parents	25	19.04	5.97			

To test hypothesis 1, a Paired Samples *t*-test was run to compare perceptions of family relationship as a risk factor of juvenile delinquency between parents and adolescents. Parent perceptions of family relationship were measured by the sum of responses to questions 8-16 on the parent/guardian survey. Adolescent perceptions of family relationship were measured by the sum of responses to questions 6-16 on the adolescent survey. As shown in Table 3, the average parent score was 19.04 (SD= 5.97) and the average adolescent score was 19.84 (SD=6.07). Although parents were more likely than adolescents to perceive family relationships as a stronger influence on juvenile delinquency, the difference between the parent and adolescent means was not statistically significant at the .05 level (*t*= -.495, df= 24). Therefore hypothesis 1 was not true. There was no significant difference between parent/guardian's and adolescent's perception of family relationships as a risk factor of juvenile delinquency.

Hypothesis 2

There will be a significant difference between parent/guardian's and adolescent's perceptions of crime/drug use as a risk behavior of juvenile delinquency.

Table 4. Results of Paired Samples t-Test on Perceptions of Crime/Drug Use

Crime/Drug Use	N	Mean	SD	t	df	Sig
Adolescents	29	9.06	3.72	-.028	28	.978
Parents	29	9.03	4.64			

To test hypothesis 2, a Paired Samples *t*-test was run to compare the perceptions of crime/drug use as a risk behavior of juvenile delinquency between parents and adolescents. Parent perceptions of crime/drug use were measured by the sum of responses to questions 17-20 on the parent/guardian survey. Adolescent perceptions of crime/drug use were measured by the sum of responses to questions 22-28 on the adolescent survey. As shown in Table 4, the average parent score was 9.03 (SD= 4.64) and the average adolescent score was 9.06 (SD=3.72). Although parents were less likely than adolescents to perceive crime/drug use as a stronger influence on juvenile delinquency, the difference between parent and adolescent means was not statistically significant at the .05 level (*t*=-.028, df=33). Therefore hypothesis 2 was not true. There was no significant difference between parent/guardian's and adolescent's perception of crime/drug use as a risk behavior of juvenile delinquency.

Hypothesis 3

There will be a significant difference between parent/guardian's and adolescent's perceptions of peers as a risk factor of juvenile delinquency.

Table 5. Results of Paired Samples t-Test on Perceptions of Peers

Peers	N	Mean	SD	t	df	Sig
Adolescents	31	4.58	1.40	1.56	30	.129
Parents	31	5.22	1.66			

To test hypothesis 3, a Paired Samples *t*-test was run to compare perceptions of peers as a risk factor of juvenile delinquency between parents and adolescents. Parent perceptions of peers were measured by the sum of responses to questions 22-23 on the parent/guardians survey. Adolescent perceptions of peers were measured by the sum of responses to questions 29-32 on the adolescent's survey. As shown in Table 5, the average parent score was 5.22 (SD=1.66) and the average adolescent score was 4.58 (SD=1.40). Although parents were less likely than adolescents to perceive peers as a stronger influence on juvenile delinquency, the difference between the parent and adolescent means was not statistically significant at the .05 level (*t*=1.56, df= 30). Therefore hypothesis 3 was not true. There was no significant difference between parent/guardian's and adolescent's perception of peers as a risk factor of juvenile delinquency.

Hypothesis 4

There will be a significant difference between parent/guardian's and adolescent's perceptions of lack of school participation as a risk behavior of juvenile delinquency.

Table 6: Results of Paired Samples t-Test on Perceptions of School Participation

School Participation	N	Mean	SD	t	df	Sig
Adolescents	32	6.59	2.28	.268	31	.790
Parents	32	6.78	2.75			

To test hypothesis 4, a Paired Samples *t*-test was run to compare perceptions of lack of school participation as a risk behavior of juvenile delinquency between parents and adolescents. Parent perceptions of lack of school participation were measured by the sum of responses to questions 24-25 on the parent/guardian's survey. Adolescent perceptions of lack of school participation were measured by the sum of responses to questions 33-40 on the adolescent's survey. As shown in Table 6, the average parent score was 6.78 (SD= 2.75) and the average adolescent score was 6.59 (SD= 2.28). Although parents were less likely than adolescents to perceive lack of school participation as a stronger influence on juvenile delinquency, the difference between the parent and adolescent means was not statistically significant at the .05 level (*t*= .268, df=31). Therefore hypothesis 4 was not true. There was no significant difference between parent/guardian's anddifference

between the parent and adolescent means was not statistically significant at at the .05 level (*t*= .268, df=31). Therefore hypothesis 4 was not true. There was no significant difference between parent/guardian's and adolescent's perception of lack of school participation as a risk behavior of juvenile delinquency.

Hypothesis 5

There will be a significant difference between parent/guardian's and adolescent's perceptions of aggression/depression as a risk behavior of juvenile delinquency.

Table 7: Results of Paired Samples t-Test on Perceptions of Aggression/Depression

Aggression/ Depression	N	Mean	SD	t	df	Sig
Adolescents	34	9.20	3.22	.954	33	.347
Parents	34	10.11	4.21			

To test hypothesis 5, a Paired Samples *t*-test was run to compare aggression/ depression as a risk behavior of juvenile delinquency between parents and adolescents. Parent perceptions of aggression/depression were measured by the sum of responses to questions 26-29 on the parent/guardian's survey. Adolescent perceptions of aggression/depression were measured by the sum of responses to questions 17-21 on the adolescent's survey. As shown in Table 7, the average parent score was 10.11 (SD=4.21) and the average adolescent score was 9.20 (SD= 3.22). Although parents were less likely than adolescents to perceive aggression/depression as a stronger influence on juvenile delinquency, the difference between the parent and adolescent means was not statistically significant at the .05 level (*t*=.954,df=33). Therefore hypothesis 5 was not true. There was no significant difference between parent/guardian's and adolescent's perception of aggression/depression as a risk behavior of juvenile delinquency.

Correlations

In reviewing the parent/guardian's and adolescent's surveys positive correlations were found. There was a significant correlation between parents knowing where there child was and listening to their child (r=.744, p< .01). Another positive correlation was found between parent/guardian having

rules for what their child can and cannot do and supporting their child's interests (r=.728, p< .01). A positive correlation was found between parent/guardian having rules for what their child can and cannot do and listening when their child speaks to them (r=.671, p< .01). A positive correlation was found between parent/guardian supporting their child's interests and listening when their child speaks to them (r=.668, p< .01). A positive correlation was discovered between parent/guardian knowing where their child is and having rules of what their child can or cannot do (r=.653, p<.01). A positive correlation was found between parent/guardian supporting their child's interests and parent/guardian listening when their child speaks to them (r=.601, p< .01). Another positive correlation was found between parents enjoying time spent with their child and parents listening when child speaks (r=.539, p<.01).

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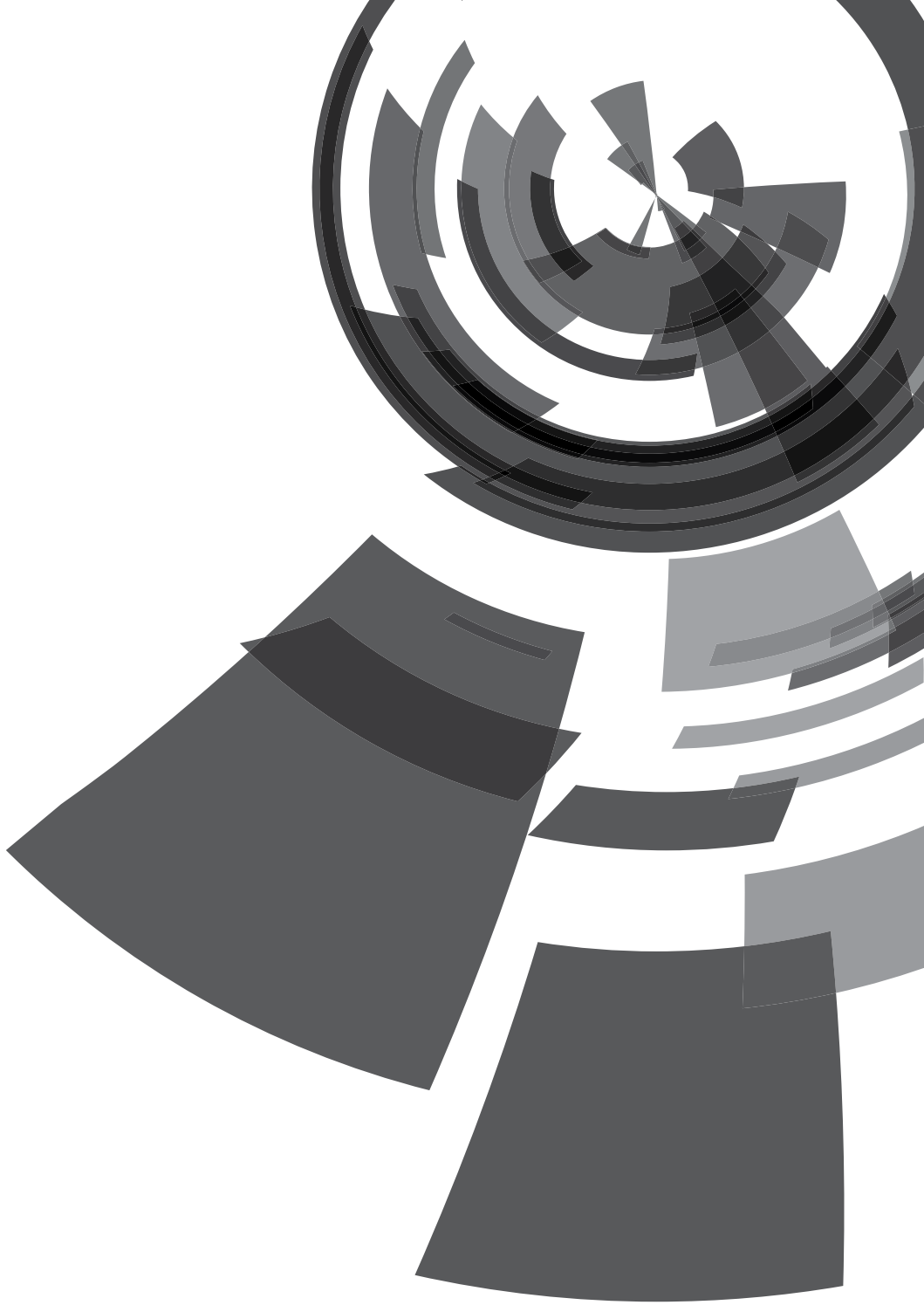

DISSEMINATION OF UNDERGRADUATE RESEARCH AT CONFERENCES AND PROFESSIONAL MEETINGS

Title: *Mossbauer Spectroscopy in the Undergraduate Laboratory*
Author(s): **Adam Decaria**
Mentor(s): **Colin Inglefield**
This research was presented at the American Physical Society Four Corners Meeting in Tucson, Arizona, October 20-22, 2011.

Title: *Phylogenic Analysis of the Unusual Chromosomal Telomeres of Drosophila*
Author(s): **Haylie Cox**
Mentor(s): **Jonathan Clark**
This research was presented at the Sigma Xi Conference in Raleigh, North Carolina, November 10-13, 2011.

Title: *The Effects of Self-Monitoring on Making Healthy Food Choices*
Author(s): **Jani Ashbaker**
Mentor(s): **Natalie Williams**
This research was presented at the Axxociation for Behavioral Analysis 37th Annual Convention in Denver Colorado, May 27-29, 2011.

Title: *Comparison of Idiomarina Bacteriophage Isolated from the Great Salt Lake, Utah*
Author(s): **Carlie Benson**
Mentor(s): **Craig Oberg**
This research was presented at the American Society for Microbiology General Meeting in 2011 in New Orleans, Louisiana, May 19-26, 2011.

Title: *Under Construction: Costume Design and KC/American College Theatre Festival*
Author(s): **Sean Bishop**
Mentor(s): **Catherine Zublin**
This research was presented at the Kennedy Center/American College Theatre Festival, Region VIII in Los Angeles, California February 7-13, 2011.

Title: *From English Learner to English Speaker – the Path of Identity*
Author(s): **Anneli Byrd**
Mentor(s): **Lauren Fowler**
This research was presented at the Critical Language Studies: Focusing on Identity in Oranjestad, Aruba, June 23-25, 2011.

Title: *The Light in the Piazza: Stage Management and KC/American College Theatre Festival*
Author(s): **Michell Casteel**
Mentor(s): **Catherine Zublin**
This research was presented at the Kennedy Center/American College Theatre Festival, Region VIII in Los Angeles, California February 7-13, 2011.

Title: *Denervation and Testosterone Changes Muscle Fiber Types in the Zebra Finch Syrinx*
Author(s): **Linsey Christensen, Lisa Allred**
Mentor(s): **Ron Meyers**
This research was presented at the Society for Comparative and Integrative in Salt Lake City, Utah, January 3-7, 2011.

Title: *The Design and Development of an Online National Dental Hygiene Board Review using a MUVE (Multi-User Virtual Enfironment) – Second Life*
Author(s): **Burke Devlin**
Mentor(s): **Kami Hanson**
This research was presented at the American Dental Hygienists Association Annual Session in Nashville, Tennessee, June 14-18, 2011.

Title: *Our Town: Costume Design and KC?American College Theatre Festival*
Author(s): **Katrina Dransfield**
Mentor(s): **Catherine Zublin**
This research was presented at the Kennedy Center/American College Theatre Festival, Region VIII in Los Angeles, California February 7-13, 2011.

Title: *Under Construction: Scenic Design and KC?American College Theatre Festival*
Author(s): **Jaime Frank**
Mentor(s): **Catherine Zublin**
This research was presented at the Kennedy Center/American College Theatre Festival, Region VIII in Los Angeles, California February 7-13, 2011.

Title: *The Effects of Twelve Hour Shifts on Performance in Pharmacy Personnel*
Author(s): **Amy Friend**
Mentor(s): **Lauren Fowler**
This research was presented at Posters on the Hill in Washington, D.C., April 11 – 14, 2011.

Title: *Posterior Clavicle Displacement of SC Joint Separation*
Author(s): **Jamie Heslop, T. Ingram, S. Norton, R. Huber**
Mentor(s): **Jordan Hamson-Utley**
This research was presented at the American Society for Clinical Laboratory Science in Atlanta, Georgia, July 26-29, 2011.

Title: *Analysis of First and Second Drops of Blood on CoaguChek XS*
Author(s): **Lauren Knudson, Daryl Blackwell**
Mentor(s): **Kara Hansen-Suchy**
This research was presented at the American Society for Clinical Laboratory Science in Atlanta, Georgia, July 26-29, 2011.

Title: *Student Directed Study: An Investigation into the Use of Podcasting*
Author(s): **Marcy Lee, Heather Tua'a, Stephanie Jenkins**
Mentor(s): **Kami Hanson**
This research was presented at the American Dental Hygienists Association Annual Session in Nashville, Tennessee, June 14-18, 2011.

Title: *Complete Peroneal Rupture in a Professional Soccer Player Managed with Conservative Treatment*
Author(s): **Levi LeFevre, Robbie Stag, Craig Allen, Dr. Andrew Cooper**
Mentor(s): **Jordan Hamson-Utley**
This research was presented at the Rocky Mountain Athletic Training Association Conference in Albuquerque, New Mexico, April 7-10, 2011.

Title: *Habitat Ecology of Pygmy Rabbits in Northeastern Utah*
Author(s): **Jennifer Schmalz**
Mentor(s): **Sam Zeveloff, Barbara Wachocki**
This research was presented at the Ninety-first Annual Meeting of the American Society of Mammalogists, A Joint Meeting with the Australian Mammal Society in Portland, Oregon June 24-29, 2011.

Title: *Perform in Classical Music Festival in Austria*
Author(s): **Nicole Sheridan**
Mentor(s): **Shi-Hwa Wang**
This research was presented at the Classical Musical Festival in Eisenstadt, Austria, August 1-17, 2011.

Title: *Novel Marinobacter-like Organism and a Related Phage Isolated from the Great Salt Lake*
Author(s): **Thomas Simon**
Mentor(s): **Craig Oberg, Michele Culumber, Matthew Domek**
This research was presented at the American Society for Microbiology National Conference in New Orleans, Louisiana, May 21-24, 2011.

Title: *Analysis of Great Salt Lake Brine Flies Expands the Phylogenetic Diversity of Wolbachia Endosymbiots*
Author(s): **Amanda Truong**
Mentor(s): **Jonathan Clark**
This research was presented at the Society for Molecular Biology and Evolution 2011 Annual Meeting in Kyoto, Japan, July 25-31, 2011.

Title: *Under Construction: Dramaturgy and KC/American College Theatre Festival*
Author(s): **Aubrey Vickers**
Mentor(s): **Catherine Zublin**
This research was presented at the Kennedy Center/American College Theatre Festival, Region VIII in Los Angeles, California February 7-13, 2011.

Title: *International Society for Language Studies Biennial Conference*
Author(s): **Catherine Byrd**
Mentor(s): **David Byrd**
This research was presented at the International Society for Language Studies Biennial Conference in Oranjestad, Aruba, June 21-25, 2011.

Title: *Religiosity Impacting Step Family Dynamics as Mediated by Dyadic Adjustment*
Author(s): **Michelle Burton and Andrew Chris**
Mentor(s): **Paul Schvaneveldt**
This research was presented at the National Council on Family Relations Conference in Orlando Florida, November 15-19, 2011.