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Cogito Ergo Sum

HONORS REVIEW

"I THINK THEREFORE I AM"

Mission Statement

Cogito Ergo Sum: Honors Undergraduate Review is a forum for undergraduate research, both guided and peer-reviewed, performed across the curriculum at SUNY Old Westbury. Undergraduate research from the Schools of Business, Education, and the Liberal Arts & Science will be celebrated in this Review to inspire greater participation in undergraduate research by students at SUNY Old Westbury and prompt a cross-fertilization of ideas. The purpose of this Undergraduate Review is to highlight the quality and the breadth of undergraduate research, both guided and peer-reviewed, via an abstract of that research and a brief selection of the research appended to the abstract. Moreover, the Undergraduate Review will be a platform for students to publicly present a sample of their research prior to official publication in a journal. Additionally, students have the opportunity to showcase academic prowess through peer-reviewed research papers. Ultimately, the Undergraduate Review will celebrate SUNY Old Westbury as a research institution, further enhancing the reputation of the College.

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Letter from Editors-in-Chief

Cogito Ergo Sum: "I think, therefore I am." René Descartes, in the most succinct way, concludes that we are alive and experiencing reality simply because we are able to think and doubt. Believing that nothing is absolute gives us the opportunity to explore beyond our imaginations, and research is that avenue that allows us to explore our doubts and reach beyond known horizons. As students at SUNY Old Westbury, we seek knowledge through research, for knowledge is a never-ending journey of learning. Therefore, this Review showcases how SUNY Old Westbury students aspire to discover new insights in the research they perform.

CES Honors Review is the first undergraduate review journal at the State University of New York at Old Westbury run by its students. This journal is an exemplar of the diligence and creativity of student research at SUNY Old Westbury as its students continue their academic journeys to new frontiers.

On behalf of the team and all the contributing authors, we truly hope you enjoy reading Volume I of our academic review journal.

Sincerely,

Maryann Johnson

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GUIDED
RESEARCH

Schizophrenia: An Assessment of High-risk Factors That May Lead to Diagnosis

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ABSTRACT

The purpose of this study was to review the literature and assess the reliability and validity of schizophrenia predictors such as socioeconomic status [SES], gender, age, race, substance abuse, and migration status. The analysis consisted of reviewing several research articles and their statistical data to determine the consistency and/or inconsistency of correlations and accuracy against past findings to see if they still hold true in today's modern world. Results were consistent with past research and showed that all predictors continue to represent a higher likelihood of psychosis diagnosis for individuals from low SES backgrounds, males, individuals between the ages of 18-32, minorities (i.e. African Americans), and migrants. Substance abuse is also consistently associated with a psychosis diagnosis; however, it may be harder to associate it as a predictor of psychosis since studies have shown that substance abuse is used as a coping mechanism against schizophrenia symptoms or is a byproduct of living in a low SES community. The potential implications of these findings are that although these predictors provide consistent reliability of diagnosis, some demographics are overrepresented and many communities overlooked. In addition, the shortage of practicing mental health professionals consequently results in overlooked/unassessed communities. Therefore, there is currently limited information gathered on the predictors of schizophrenia. This may also alleviate the over-representation of one group in the area of psychosis.

INTRODUCTION

According to the *Diagnostic and Statistical Manual of Mental Disorders 5th edition* (*DSM-V*), schizophrenia spectrum disorders are defined as "abnormalities in one or more of the following five domains: delusions, hallucinations, disorganized thinking, grossly disorganized or abnormal motor behavior, and negative symptoms" [3]. In contrast, the criteria for establishing a diagnosis of Schizophrenia within the *International Classification of Diseases Tenth Edition* (ICD-10) is defined as "a major psychotic disorder characterized by abnormalities in the perception or expression of reality" [3]. Globally, the schizophrenia prevalence rates from 2010 to 2017 for schizophrenia were highest in China with 468,551 cases in 2013; from 1990 to 2016, schizophrenia cases rose from 13% to 21% (8% increase in prevalence) and typically affected individuals that were 25-54 years old [10,16].

Schizophrenia was first described and identified by Dr. Emil Kraepelin, a German psychiatrist during the 19th century, as *Dementia Praecox;* however, it was later renamed to schizophrenia by Eugen Bleuler, a Swiss psychiatrist, in 1908 [5]. Before the 19th century, many symptoms that would fall within the criteria for schizophrenia were diagnosed as "mania or generic insanity," or identified with the concept of neurosis by Karl Friedrich Canstatt in 1841 [18]. He emphasized this concept as a disease of the brain [8]. By 1913, diagnosis of mental disorders was dichotomized by psychoses as "the result of somatic illnesses, therefore a *process"* and by neuroses to "have psychological biographical causes, therefore *develop"* [8]. Dichotomous diagnoses of psychosis could no longer be relied upon as psychiatrist Karl Jaspers in 1913 had historically explained,

> "Pathological symptoms are layered like an onion, with degenerative symptoms (primarily the psychopathies, but also Kraepelin's manic-depressive insanity)

> forming the outmost layer, moving inwards to the process symptoms (schizophrenias), and finally, the innermost layers comprising organically based symptoms. The deepest layer reached in the course of examining an individual case is decisive. What initially appears to be a case of hysteria turns out to be multiple sclerosis, suspected neurasthenia is actually paralysis, melancholic depression a process." [19]

The generic understanding of psychosis would forever be changed with a thorough investigation into the precise classification of patient symptoms.

Although the advancements of clinical diagnostic criteria have increased the accuracy and precision in diagnosing schizophrenia (e.g. recognizing it as a degenerative mental disorder), the diagnosis can still be rather subjective. This could in part be due to similar and overlapping symptoms within diagnosis criteria. Trierweiler et al. (2006) alludes to potential wide-range differences in cultural norms for different ethnic groups [34]. Professionals would first have to consider the ethnic and cultural background of the patient as part of their medical history prior to making any concrete diagnostic conclusions. In the case of substance abusers, for example, mental health professionals may find it challenging to determine whether or not the symptoms are due to the disorder or drug abuse that may be a part of one's cultural norms or environment (i.e. growing up in statistically higher drug and crime rated areas, drug use being a norm among family members) [34]. More importantly, the situation can be further compounded by the potential comorbidity of several chronic mental disorders that can make the etiology of the disorder more elusive for a professional.

Psychosis is defined as disorganized speech ("word salad"), disorganized thoughts, and having a lack of connection with reality. Contrastingly, personality disorders are categorized by

specific behaviors or thoughts that are considered outside of social norms. A mood disorder is typically a disturbance of one's life due to an extreme change in mood. A diagnosis of a mood disorder can accompany psychosis so long as the psychosis occurs simultaneously during mood episodes. Thought and delusional disorders can also be present without comorbidity of psychosis.

Schizoid and schizotypal classifications are both personality disorders. Individuals diagnosed with schizoid have no interest or desire for social interaction or connection; they are typically withdrawn and voluntarily isolate themselves. Whereas, individuals diagnosed with schizotypal have symptoms of the schizoid disorder in addition to "magical thinking" or bizarre thoughts and behaviors; this most likely leads to psychotic disorders like schizophrenia, schizoaffective disorder, and schizophreniform.

Schizophrenia is a chronic degenerative disease that is characterized by distorted reality; family members who have been diagnosed with schizophrenia are more likely to have a relative with schizoid, schizotypal, or schizoaffective disorder [6,20]. Because the schizotypal disorder is a personality disorder, schizophrenia can be characterized by psychosis with the presence of schizotypal symptoms. Moreover, these symptoms should be present for 6 or more months to be classified as schizophrenia, whereas schizophrenia symptoms lasting up to a month or less are characterized as brief psychotic disorder [3].

Schizoaffective disorder is classified as a mood disorder, such as major depressive disorder (MDD) or bipolar disorder, with schizophrenic symptoms. This disorder can be considered as a dominant diagnosis when the dominant schizophrenic symptoms last two weeks or more without the presence of the second diagnosis (bipolar symptoms) [3]. The later appearance of bipolar symptoms would lead to a schizoaffective disorder diagnosis. Finally,

schizophreniform is characterized by schizophrenic symptoms that only last between one to six months [3].

Typically, diagnoses that fall within the schizophrenia spectrum disorders include the addition of one or several of the following positive symptoms which can surface at the onset of psychosis: delusions and/or fixed beliefs that "are clearly implausible and not understandable to same-culture peers and do not derive from ordinary life experiences," hallucinations that are sensory perceptions occurring without any prior stimulus (auditory is the most common), and disorganized speech and abnormal motor behavior (i.e. catatonia, motor immobility or hypermobility, and lack of movement in otherwise non-natural positions) [3]. In contrast, negative schizophrenic symptoms are defined as the *decrease* in or *lack* of normal levels of sociability, emotions, interest, speech, or desires [3]. Experiencing these positive and/or negative symptoms can also be accompanied by stress, depression, agitation, and confusion, which makes schizophrenic individuals more vulnerable towards social-emotional fragilities [3]. The latter point is critical, as adolescents who display schizophrenic symptoms by age 17 and younger have diminished recovery outcomes, whereas adults that are 18 years and older are expected to have more promising recovery. This could be attributed to "longer length of initial hospital stay, more readmissions and hospital days per year than people aged 18 or older" [25]. The *DSM-V* diagnosis criteria also note that early onset of psychosis typically "has been seen as a predictor of worse prognosis" and that "features typically emerge between late teens and mid-'30s; onset prior to adolescence is rare" [3].

LITERATURE REVIEW/HYPOTHESIS

Economic status tends to play an important role in predicting whether individuals might be at a higher risk of being diagnosed with schizophrenia. Research implies that individuals typically in low SES or urban areas are at higher risk for "schizophrenia and related disorders" [3]. The results from a population-based study showed that "poorer residential areas and SES were risk factors for schizophrenia," despite having experimental controls such as educational and occupational status of parents [36]. Another population study conducted from 2004 to 2010 found that "schizophrenia was found to be more common in low-income individuals compared to [high]-income individuals, [where] 1.23% of low-income participants were diagnosed with schizophrenia within six years, [while only] 0.26% of [high]-income participants were diagnosed" [21]. Charlson et al. (2018) reported that "the large burden of schizophrenia experienced in lower and upper-middle-income countries is around four times the burden experienced by high-income countries;" this was supported by the results in McGrath et al. (2004), as it was noted that urban communities reported higher rates of schizophrenia ($p = 0.02$) than mixed urban-rural communities [10,23].

Men are also at higher risk for a schizophrenia diagnosis than women [3]. Aleman et al. (2003) noted that when "only high-quality studies were included," the incidence risk ratio for men was 1.39 (95%) in 11 studies and 1.42 (95%) in 49 studies when compared to women [1]. The results in Werner et al. (2007) indicated that the incidence of schizophrenia was higher among male subjects at 0.87%," while females had a 0.64% incidence [36]. Cohen et al. (2014) found that in their study of speech deficits that patients with schizophrenia were more likely male that had severe positive symptoms [12]. The meta-analysis conducted by Aleman et al. (2003), also reported that "the studies that minimized sampling bias still show[ed] a considerable sex

difference in schizophrenia incidence," and that a smaller sex difference in diagnoses that predated 1980 were noted [1]. Lee et al. (2018) further reported higher numbers of cases in males from low economic status communities between the ages of 18 - 44 years old [21]. The global prevalence of schizophrenia details that the onset of schizophrenic symptoms and its subsequent diagnosis occur around the age of 40 [10]. Furthermore, Fisher et al. (2014) stated that children around the age of 11 with psychotic symptoms have a higher risk of developing the disorder by the age of 38 [15].

Although men have higher incidence rates, women are also likely to have onset of symptoms earlier than men by three to five years. Moreover, while Charlson et al. (2018) noted that individuals were typically affected between the age of 25 - 54, Li (2016) reported that the onset of symptoms in men was typically between the age of 21 - 25 and in women was between the age of 25 - 30 or after the age of 45 [10,22]. Ochoa et al. (2012) also supported that "women seem to have two peaks in the age of onset of the disease: the first after menarche and the second once they are over 40" [27]. Additionally, women "make up 66 - 87% of patients with onset after the age of 40 - 50 years old" [22].

A number of studies have proposed that African Americans are more likely to be diagnosed with schizophrenia, substance-related use/abuse disorders, shorter length of hospital stays, and are less likely to be diagnosed with mood disorders compared to white and Hispanic patients [14]. Cohen et al. (2013) conducted a study to explore this idea by attempting to determine the differences in negative symptoms, primarily diminished expressivity, among ethnicities by gathering recorded samples from several participants with schizophrenia or mood disorders [12]. They reported that "the schizophrenia group was significantly younger, more likely to be African-American, and had more severe bizarre-behavior symptoms;" 67% of African

Americans had schizophrenia, while 30% of Caucasians had schizophrenia [12]. Another study by Swartz and Horner (2004) that assessed military veterans underlined that "a greater proportion of African Americans had a diagnosis of schizophrenia spectrum disorders and drug use disorders, fewer were diagnosed with [posttraumatic stress disorder] (PTSD) or bipolar disorder," where 48% of African Americans, compared to 17% of Caucasians, were diagnosed with schizophrenia [9].

Neighbors et al. (2003) also sought to determine the rates of schizophrenia among African Americans and whites; however, they assessed differences in diagnoses of schizophrenia, schizoaffective disorder, bipolar disorder, and major depression [26]. Their results indicated that African Americans displayed higher percentages of schizophrenia (44%) compared to Caucasians (32%), but had lower percentages of bipolar disorders (5.4%) compared to Caucasians (14.3%) [26]. These results were supported by Swartz & Horner (2004) and Delphin-Rittmon et al. (2015) [9,14].

Trierweiler et al. (2006) tested for differentials in diagnosis based on doctor-patient race differences [34] by sampling 292 low-income adults primarily of African Americans (72% African Americans and 28% non-African Americans). They reported that when African American and non-African American clinicians were assigned to assess patients already diagnosed with schizophrenia or major affective disorder, non-African American clinicians were more likely to diagnose African American patients with negative symptoms and thought disorders. There was no evidence that the clinician's ethnicity influenced the rate at which patients were diagnosed with schizophrenia since they all had a formal diagnosis pre-study. These results suggest differences in symptom attribution by race, but further study is required to determine whether the race of the doctor affects this symptom attribution.

The comorbidity that exists frequently with schizophrenia cases could suggest that substance use/abuse might temporarily mask the symptoms faced by individuals with chronic comorbid illnesses. In a study surveying 40 men with/without comorbid diagnoses, Altunsoy et al. (2015) noted that those without comorbidity were more likely to have mundane jobs and formal education; thus, failing to make the connection between a lower quality of life and substance abuse as an equitable contributing factor is difficult [2]. In a qualitative study by Asher and Gask (2010), several participants diagnosed with schizophrenia and drug use were asked to assess their drug use habits [4]. Their results suggested that drug use was influenced by an external factor that could be associated with their diagnosis.

> "A subgroup of people who have severe mental illness and who feel alienated from conventional services and society, use illicit substances, have unstable impoverished social circumstances, and see little chance of ever gaining employment." [4]

This can also indirectly relate that low SES could be a predictor of schizophrenia. Pampel et al. (2010) noted that those in low SES communities "struggle to make ends meet, have few opportunities to achieve positive goals, experiences more negative life events such as unemployment, marital disruption, and financial loss, and must deal with discrimination, marginality, isolation, and powerlessness" [28]. These stressors could promote drug use, and possibly explain the coupling of psychosis and drug use.

Of the several drug substances that were screened, there was a correlation between cannabis use and schizophrenia diagnosis. Hutchinson's (2019) etiology analysis of psychoses noted that "there is strong evidence that the use of certain substances, particularly cannabis, is associated with an increased risk of psychosis" [24]. Sarrazin et al. (2015) results would support

this analysis as their participants who were diagnosed with cannabis abuse or dependence according to the *DSM-V* criteria had preexisting schizophrenia diagnoses [32].

Past literature has also suggested that migration status could serve as a predictor of schizophrenia. Higher rates of psychosis among migrants could potentially be attributed by frequent discrimination, violence, isolation, assimilation (or lack thereof), and other aspects like low ethnic density areas [13,24]. McGrath et al. (2004) systematic review noted several migrant studies from Germany, Sweden, the United Kingdom, and the Netherlands, and reported that "migrant groups displayed elevated incidence of schizophrenia compared to their native-born population" [23]. Additionally, the Migration Report 2017 highlights from 2000-2017 displayed that Asia came second in-migrant population (67,259) and the GBD Collaborative network confirmed that Asia had the highest number of schizophrenia cases from 2010-2017, suggesting high numbers of reported cases of schizophrenia amongst the high numbers of migrants [16].

Factors associated with a higher risk of schizophrenia can include SES, gender, substance abuse, and migration status. It is hypothesized that although SES, gender, and substance abuse tend to be associated with a diagnosis of schizophrenia, patient-doctor cultural differences also appear to affect how symptom attribution is considered. Thus, migration status should not be used as an indicator for the risk of schizophrenia.

METHOD

The inclusion criteria for this study required that the studies were published between the years 2000 to 2020 and included patient socio-demographic information (i.e. SES, age, gender, ethnicity, substance abuse, and migration status) of individuals diagnosed with schizophrenia. The United Nations migration reports were also used to cross-reference migration and schizophrenia case numbers with the data. Finally, the *DSM-V* was used to ensure the

consistency of schizophrenia symptoms reported in the included articles. The exclusion criteria consisted of studies published before 2000 or studies published between 2000 to 2020 that failed to include any/clear patient demographic information.

Notably, *Google Scholar* and *PsychInfo* were the databases that were employed to gather the necessary peer-reviewed sources. However, world databases, such as the GBD collaborative network, were also used to assess global cases of schizophrenia through the years 2010 to 2017 and then compare/contrast those cases with published reports (e.g. the World Migration Report)[16]. In each of the databases used, the following *keywords* were used: *"schizophrenia," "predictors," "age," "SES," "gender," "race," "substance abuse," "migrant status," and "diagnosis."*

The *keywords "schizophrenia," "predictor,"* and *"age"* in Google Scholar returned 27,800 articles, while PsychInfo returned 1,342 articles. Six articles were relevant from Google Scholar and three articles were relevant from PsychInfo, and two articles were used from Google Scholar. With the *keywords "schizophrenia diagnosis"* and *"socioeconomic status,"* Google scholar returned 27,900 articles, with five that were relevant, while PsychInfo returned 33 articles of which four were relevant. A total of four articles were used for analysis, of which three were from Google scholar and one was from PsychInfo.

The *keywords* "*schizophrenia diagnosis"* and *"gender"* returned 237,000 articles on Google Scholar, and 324 articles were returned from PsychInfo. Ten articles were relevant from Google Scholar, and two articles were relevant from PsychInfo. A total of four articles were used for analysis that was from Google Scholar.

For racial assessment, the *keywords "schizophrenia diagnosis"* and *"race"* returned 35,800 articles from Google Scholar, and 64 articles were returned from PsychInfo. Eight articles

were relevant from Google Scholar, and seven articles were relevant from PsychInfo. Two articles were taken from PsychInfo, and four articles were taken from Google scholar for a total of six for analysis.

Google Scholar returned 206,600 articles for the *keywords "schizophrenia diagnosis"* and *"substance abuse"* with five that were relevant, while PsychInfo returned 172 articles with three that were relevant. One article was used from PsychInfo and two articles from Google Scholar for a total of three substance abuse articles used for analysis.

Finally, the *keywords "schizophrenia diagnosis"* and *"migration"* returned 17,700 articles from Google Scholar and one article from PsychInfo. All relevant articles were used as three were produced from Google Scholar and one from PsychInfo. Twenty-four articles in total were used, two manuals to assess schizophrenia diagnosis criteria (*i.e., DSM-V*, and the *ICD-10*), and three databases to cross-reference case and migration prevalence (GBD, World migration report) (**Figure 1)** [16].

RESULTS

The world statistics on migration reported that Europe and Asia were the leaders of migration between 2000 to 2015, with Europe having 70-80 million migrants [29]. The GBD reported that China showed the highest numbers of schizophrenia cases between 2000 and 2017 with over 340,000 cases reported in 2011 [16]. Notably, the World Migration reports show that males were more likely to migrate than females [29]. Migrants also tended to be between the ages of 25 to 44 years old for both genders [29]. These findings are also consistent with the ages that schizophrenia diagnoses are often made (i.e. with most cases reported between 18-44 years old). However, most migrants were from higher income brackets (i.e. a necessary means to electively migrate in contrast to a refugee); thus, this particular wealthy migrant group would not

correspond with the SES predictability factors reported in the literature [29]. Furthermore, individuals that come from low SES backgrounds are less likely to migrate due to cost concerns. It was hypothesized that although age, gender, substance abuse, ethnicity, SES, and migration status were all acceptable potential predictors for schizophrenia based on previous research studies, migration status could not be used as a valid predictor.

According to the World Migrations statistics and case reports of schizophrenia, high numbers of migrants closely correspond with high cases of schizophrenia. Lee et al. (2018) and Werner et al. (2007) reported that those that live in low SES are at higher risk to be diagnosed with schizophrenia [21,36]. Lee et al. (2018) reported higher rates of cases among men and women from low SES communities out of 15,098 low-income participants with 1.23% of cases being in low SES versus 0.26 % in non-low SES communities [21]. Werner et al. (2007) reported that out of 69,384 participants in either low or non-low SES communities, low SES communities were found to have 0.92% of cases, while 0.69% of cases were found in non-low SES communities [36]. Minorities typically make up low SES populations; however, further study should consider the validity of the past literature due to the potential demographic biasing of African Americans. In these studies, the African Americans comprised a small part of the sample population in the areas where the studies took place. Further investigation of whether the sample size was statistically meaningful in terms of effect, size, and power analyses is required to validate this claim. Substance abuse also appears to be a coping mechanism for individuals with schizophrenia or in extenuating circumstances, rather than an indicator of schizophrenia. Lastly, studies of out-group diagnosticians when compared to their in-patient diagnostician, are lacking in the literature to best address this demographic issue of low SES community diagnoses of psychopathology. **Figure 2** displays the percentage of relevant articles versus the total articles

returned by each keyword search in the Google Scholar database. The total number of relevant articles was divided by the total number of articles returned and then multiplied by 100. Out of 206,600 results for keywords "*schizophrenia diagnosis*" and "*substance abuse,*" less than one percent (0.002%) of the articles that were relevant to this analysis conducted herein. The keywords "*schizophrenia diagnosis*" and "*gender*" also resulted in a return of 237,000 total articles with less than one percent of them being relevant articles (0.004%). The keywords "*schizophrenia,*" "*predictor,*" "*age,*" "*schizophrenia diagnosis,*" and "*race*" returned less than one percent (0.022%) of relevant articles.

Figure 3 also represents the percentages of the total number of articles returned, and of those that were relevant for each keyword search in PsychInfo. Much like Google Scholar, the percentages of relevant articles were low in relation to the total number of articles returned. However, PsychInfo's total number of articles returned was significantly lower than Google Scholar. The keywords "*schizophrenia diagnosis*" and "*socioeconomic status*" returned 33 total articles, whereas "*schizophrenia diagnosis*" and "*race*" returned 64 total articles, which represented 12% and 10% of relevant articles obtained, respectively. One hundred percent of the returned articles for "*schizophrenia diagnosis*" and "*migrant status*" were used. However, it is suggested to exercise caution here as there was only one article to take into account that came from PsychInfo for these keywords.

In both databases the keywords "*schizophrenia diagnosis*" and "*migrant status*" resulted in the least amount of relevant articles confirmed. This suggests a need for further study into schizophrenia and migrant status as a potential predictor/risk factor. It is interesting to note that despite Google Scholar returning more articles than PsychInfo, there were fewer relevant articles obtained from this search engine/database. This may substantially lower the amount of

overwhelming and irrelevant material when searching. PsychInfo produced lower numbers of articles returned, but the number of relevant articles obtained was greater than Google Scholar. This suggests that PsychInfo may be a more appropriate search engine/database for finding peer-reviewed scholarly articles on this topic.

DISCUSSION

The etiology of schizophrenia continues to be assessed through research, and the world health reports further emphasize the importance of research-guided diagnoses. With proper research-guided diagnoses, patients can be correctly matched with the necessary mental health services sooner; this, in turn, would lead to a higher likelihood of a positive quality of life outcome. Individuals in low SES communities are at higher risk for developing schizophrenia, while also facing a lower amount of mental health services within their communities. A behavioral workforce study conducted in 2018 reported that "of the 3,135 counties in the United States, 1,522 had at least one psychiatrist of the four types [(i.e. General, child/adolescent, geriatric and addiction psychiatrists)] included in this study (48.5 %)" [7]. Additionally, the World Health Organization (WHO) reports "in 2014-2016, low-income countries have 0.1 psychiatrists…per 100,000 people "[38]. "The rate of psychiatrists in high-income countries is 120 times greater" [37]. These statistics should indicate the real need for psychiatrists and the greater need for them to work with patients in low SES areas.

Individuals of all ages would benefit from a greater workforce of psychiatrists serving adolescents and the elderly as they are at increased risk for developing a schizophrenia diagnosis. The low rates of psychiatrists in addiction services and the large overlap of substance use/abuse being diagnosed comorbid with schizophrenia places the patients at an additional disadvantage. Practicing mental health specialists numbered 836 across the United States, and although the

WHO reported less than 1% of practicing psychiatrists in 39% of countries, 24% were absent. This makes the dataset difficult to interpret and gauge any meaningful information both globally and in the United States [7,37]. Although the research reviewed did not indicate substance use/abuse as a cause for schizophrenia, patients with psychoses were more likely to participate in drug use to cope with their symptoms and could perhaps benefit from an addiction psychiatrist.

Males are more likely to be diagnosed with schizophrenia compared to women, and further study would be needed to address these gender disparities when diagnosing schizophrenia. African Americans are also diagnosed at higher rates with schizophrenia than other ethnic minority populations; however, Hutchinson reports finding "strong evidence that outcomes…were substantially worse for patients of black Caribbean ethnicity and worse or no different for patients of black African ethnicity, compared with white British" [24]. Should further research continue to suggest higher rates of schizophrenia in African Americans, the findings by Hutchinson may be a starting point in determining the reason for this overdiagnosis since outcomes for African Americans are worse than for their Caucasian counterparts [24].

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Table 1. Illustrates the keyword comparisons between the databases used in the study.

Database	*Keywords used*	*Total findings from search*	*Total relevant findings*
Google Scholar	*"schizophrenia" "predictor" "age"*	27,800	6
	"schizophrenia diagnosis" "socioeconomic status"	27,900	5
	"schizophrenia diagnosis" "gender"	237,000	10
	"schizophrenia diagnosis" "race"	35,800	8
	"schizophrenia diagnosis" "substance abuse"	206,600	5
	"schizophrenia diagnosis" "migrant status"	17,700	3
PsychInfo	*"schizophrenia" "predictor" "age"*	1,342	3
	"schizophrenia diagnosis" "socioeconomic status"	33	4
	"schizophrenia diagnosis" "gender"	324	2
	"schizophrenia diagnosis" "race"	64	7
	"schizophrenia diagnosis" "substance abuse"	172	3
	"schizophrenia diagnosis" "migrant status"	1	1

Figure 2. Illustrates the percentage of relevant articles found for each word search from Google Scholar.

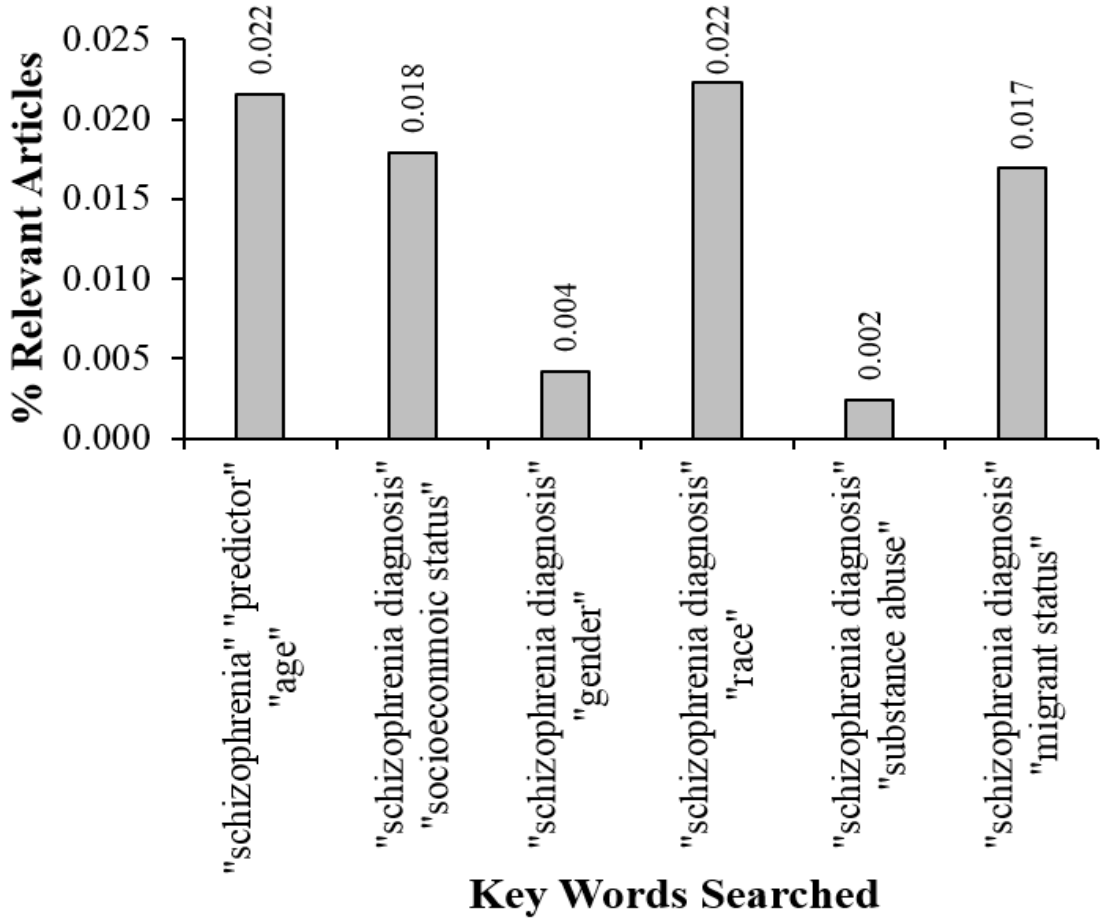
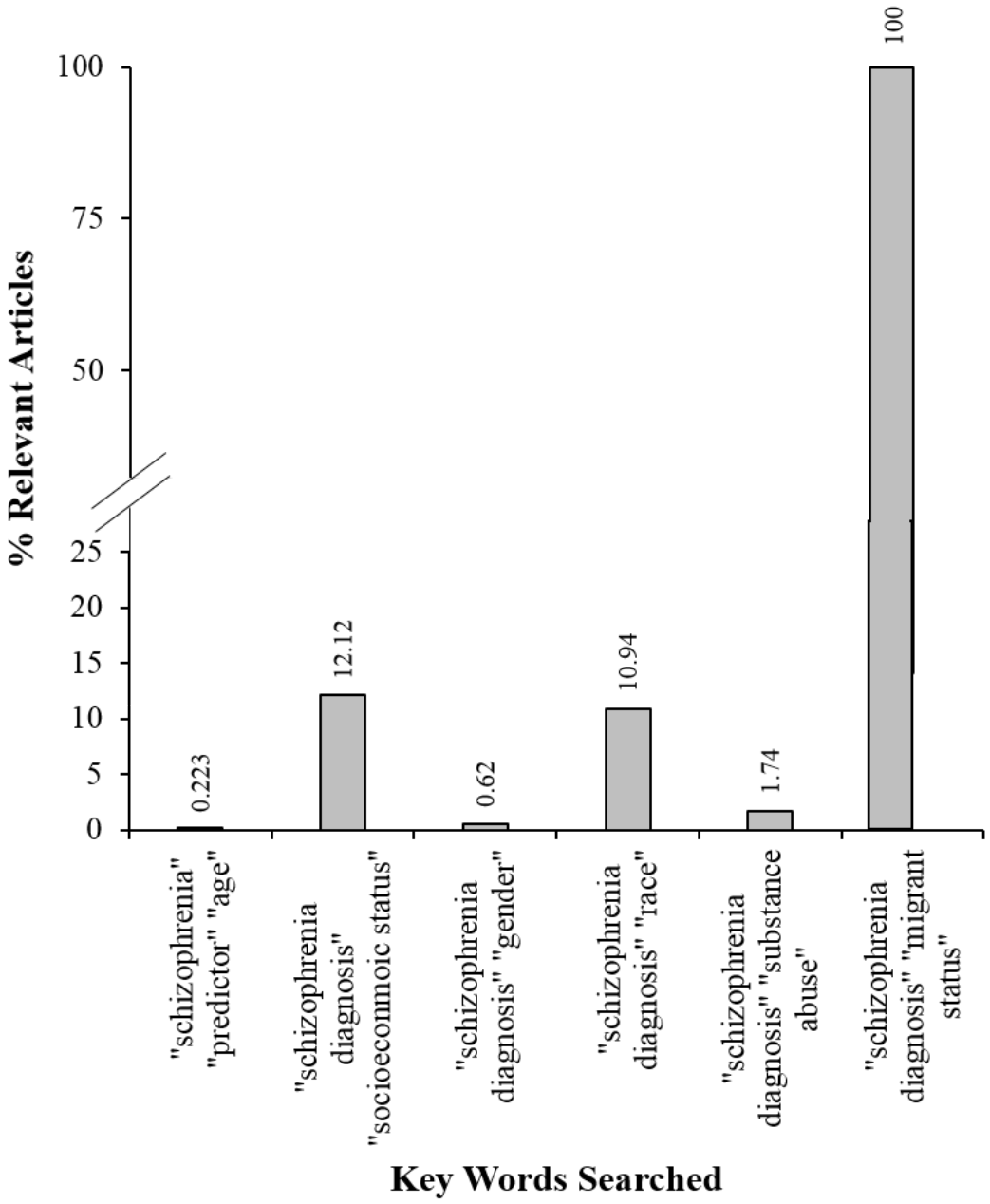


Figure 3. Illustrates the percentage of relevant articles by word search from PsychInfo.



Religiosity/Spirituality and Academic Performance in College Students

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ABSTRACT

With several platforms in place to ensure the development and success of students, religion and its teaching have been proposed to provide the necessary traits and behaviors associated with reaching that success. Spirituality provides many potentially constructive behaviors and traits for academic success as well. This study sought to find any correlational relationship between religiosity/spirituality and academic performance of college level students, as well as if any groups proved potential significance. Through construction of several standardized surveys assessing participants' level of religious involvement, spirituality, grade point averages, and whether they practice deep or surface study habits, this potential correlation was assessed among college level students. A total of one hundred and ten New York State College students were assessed during the Fall 2020 and Spring 2020 semesters. Results indicate no significant correlation between religiosity/spirituality and academic performance. In addition, groups by gender, college level (juniors and seniors), and ethnicity (black and white) showed no significance with respect to religiosity/spirituality and academic performance.

INTRODUCTION

Some important aspects of young adults' lives include educational performance and religion. These two variables play important roles in personal development into higher learning, so it is not surprising many studies have been done to find correlations between them. Religion plays a large role in the structure and moral aspects of peoples' lives. Therefore, understanding how it could potentially contribute to higher learning is important. Religion consists of believing and/or actively participating in religious activities. This could include mainstream religions such as Christianity or Judaism or a spiritual connection, since religion and spirituality are not exclusively interchangeable and will be treated as such for this study. Having a stronger connection with religion could result in higher levels of academic achievement and improved study habits such as engaging in Deep study vs. Surface study [9].

Unfortunately, much of the research conducted has focused largely on secondary school youth rather than college level students and beyond. Although the results from the studies focused on secondary school youth held consistent results, religion and academic performance still positively correlate in the few studies whose participants were above the age of 18 [3]. High School students who reported being very religious were more likely to complete their bachelor's degree than those who were not; additionally, higher grades in school were typically reported by students who had stronger religious convictions [6,11]. Although much of the research has focused on several variables, the inconsistency of the consideration of these variables should be noted. Parental involvement, religious activity attendance, and socioeconomic status should also be considered in determining the correlation between religion and academic performance since they all could affect a students' educational success.

This study seeks to assess the correlation between religiosity/spirituality and academic performance in college level students by categorizing their study habits and GPA. Group comparisons are used to detect any significant differences between gender, academic level, and ethnicity with respect to religiosity/spirituality and academic performance. Although religion and spirituality are not interchangeable, it is still hypothesized that with greater self-reports of spirituality, students are more likely to have better academic performance. Another task not reviewed in these previous studies was group comparisons; therefore, this study will compare groups by gender, academic level (juniors and seniors), and ethnicity (black or African American and white).

LITERATURE REVIEW

Religion: Religiosity & Spirituality

There are many positive and useful traits that religion can instill into its followers, such as asense of belonging, distinguishing between right and wrong, discipline, and the desire to succeed in life with a community of fellow theists to support them. It's not uncommon to consider that the ethical teaching of a religion could aid in several other aspects of a person's life. Regardless of what religion one identifies with, one can agree that the overarching messages of religion have positive connotations. Religion could provide comfort, stability, and sometimes clarity. William H. Jeynes suggested that religion could be responsible for instilling a religious work ethic and has a high probability of ingraining an internal locus of control. He includes that religion also teaches against harmful behaviors that may not facilitate positive life choices [3]. Furthermore, Marie Good and Teena Willoughby's study provided the effect of high religious activity involvement and substance abuse and found that substance abuse was significantly lower for participants who reported frequent religious service attendance [2].

Although religion and spirituality are commonly used synonymously, they do not have all the same defining characteristics. Religion tends to be used in partnership with the religion one identifies with, and although religious individuals can be spiritual, spirituality does not need to be tied to a religion, like agnosticism. The belief in a deity or a higher power simultaneously belongs to spirituality and religion. Prior studies, like the study conducted by Toldson and Anderson, chose to define religious belief by self-reported level of religious convictions, including questions on frequency of attending religious services and the importance of religion within their lives [11]. Other tests such as the National Educational Longitudinal Survey (NELS) also included questions on the religiosity level of the participants [6]. The Duke University Religion Index (DUREL) assesses religious faith by including items on the frequency of religious attendance and how much importance religion has in one's life decisions [9].

Throughout these studies, students were only grouped by grade cohorts or religiosity/spirituality levels. Some studies addressed outside factors like parental involvement, socioeconomic level, or peer connections, but no groups looking to be assessed in this study were compared against one another in any previous literature.

According to the Pew Research Center's 2020 statistics, females were reported to have a higher belief in god at 69%; additionally, more females reported feeling spiritual peace at 64%. In a study done by Raffy R. Luquis, Gina M. Brelsford, and Liliana Rojas-Guyler, of the 960 college students that were evaluated, females were reported to have higher levels of spiritual experiences as well as higher levels of religiosity in comparison to men [5].

Academic Performance

Several approaches have been set in place to assist the success of students in most levels of education, including No Child Left Behind and Head Start [3]. Through these programs,

different aspects of education are highlighted to ensure their success. Parental involvement, uniforms, and multicultural teaching are some of the focuses of these programs because they could play a crucial part in the development of a student's academic career [3].

Academic performance is easily evaluated by grades or awards for noticeable achievements. Standardized test grades, and the frequency of disciplinary actions can also contribute to the understanding of a student's academic performance. Another possible indicator of academic performance would include the study habits of students. The Approaches and Study Skills Inventory for Students (ASSIST) measures the style of study habits by assessing deep, strategic, or surface approach studying. Deep approach correlates to understanding content and active learning, strategic approach correlates with habits such as discipline, time management, and the desire to excel, and surface approach typically relates to memorization, and passive learning [9].

Monitoring the Future is a questionnaire that also attempts to assess academic success via a Likert scale that allows the participants to reflect on their ability for academic success, but interestingly some studies chose to include some other aspects in academic success, such as peers' attitudes and degree attainment [11]. Peer attitudes, simply measured by the importance of peer's desire to do well, can be another potential indicator of academic performance [2]. Overall, there are several aspects that can contribute to academic performance and should be considered when understanding performance in higher education, since variables such as parental involvement may be less salient for campus or more mature students.

Academic performance typically is lower in minority students. For example, the average high school GPA for African American males is the lowest at 3.50, while African American females showed an average GPA of 3.61 [4]. These were both still below the average GPA for

white students with a reported average GPA of 3.78. First college term GPAs for African American males was still on average the lowest at 2.91, whereas average GPAs for African American females was slightly higher at 2.98. However, white students still reported the highest with 3.31 for males and 3.35 for females [4]. In a study performed by Donald F. DeMoulin and Martin, sampling 142 junior and senior college students, mean GPA scores were slightly higher for college seniors (3.06) than they were for college juniors (2.94) [1].

Religiosity/Spirituality & Academic Performance

With all the aspects considered in the development of academic performance, religion is consistently thought to be a valuable part. It is hypothesized that college students who report being religious will more likely have higher GPAs and better academic performance. Academic performance benefits significantly from the positive habits that are frequently associated with religion. In Jeynes' meta-analysis, he notes the achievement gap being significantly lessened when all aspects of religion (faith, religious school, orientation) are present in a student's life [3]. Religious service attendance was also positively correlated with higher grades [2]. These positive correlations suggest religion does typically produce students who excel in education, and typically have less disciplinary actions taken and engage in little to no negative activities. Disciplinary problems were negatively correlated with religious participation, suggesting that students who report importance in religion also reported few necessary disciplinary actions [11]. Substance abuse was also negatively correlated with religious activity [2].

In Jessica Schubmehl, Stephen Cubbellotti, and William Van Ornum's study, the Index of Core Spiritual Experience (INSPIRIT) was used to assess spirituality in sophomore and junior college students [8]. Students who scored high on the INSPIRIT were positively correlated with higher GPAs [8]. Assessment of variables such as locus of control, parental involvement, and

religiosity showed a positive correlation with degree attainment [6]. This suggested that even when the variables associated with spirituality are measured, the probability that the teachings of religion or spiritual belief aided in positive skills is high. Although important, this study was conducted amongst sophomores and juniors attending a private religious institution. Despite this, it is clear that religion can serve as a positive foundation for the academic success of secondary schools and higher learning students.

Overall, whether one identifies as religious or spiritual, past literature supports the idea that with religiosity and/or spirituality, positive attributes such as community involvement, parental involvement, less harmful decision making, and work ethic aid in academic performance for students. Ability to complete undergraduate degrees and high locus of control may also be associated with positive behaviors that increase academic performance.

METHOD

Design

To evaluate the hypothesis, a correlational study and independent t-tests were conducted for three different groups to compare their religiosity/spirituality to their academic performance. In addition, gathering surveys from participants from the Spring 2020 semester, as well as from the Fall 2020 semester categorizes this study as longitudinal. Morling defines a correlational study to include variables that are measured to further test the relationship between them [7]. She categorizes independent t-test as determining "whether the difference between two group means in an independent groups design is statistically significant" [7].

Religiosity is defined by self-reports of religious affiliation and religious event attendance or frequency of places of worship. Although used with religiosity, spirituality is defined as a feeling of connection with a higher being that is not in line with mainstream religious affiliations

such as Christianity, Judaism, Islam etc. This is due to the fact that those who are spiritual may not necessarily be religious or identify with a religion. Questions to assess both options are present within the second section of the survey. Academic performance is the second variable and is defined by reported GPA, and study habits consisting of deep, strategic, or surface level study. All possible options for this subcategory are also assessed within the survey.

Participants

Participants in this study were college students from the New York area and this was the only criteria necessary to participate. In total, 110 participants took part in the survey. Seventy-seven percent of them were female, and majority of those reported being either juniors (41%) or seniors (40%). Both white and black participants made up 30% of the sample while Hispanic or Latino and Asian/Pacific islanders made up the next largest portion at 15% and 10%, respectively.

Out of 103 participants, the average age was reported at 21 (M = 21.97, S.D. = 3.300). The oldest participant reported was 38, while the youngest was 17 across the Spring 2020 and Fall 2020 semesters.

Those who took this survey were also asked about their religious affiliation. 49% were affiliated with Christianity, 15% reported to be non-religious, while 8% and 3% of the student sample affliated with Islam and Judaism, respectively. With respect to the representative religious demographics of New York State, participants in this study show very similar demographic makeup. Higher reports of Judaism than Islam are seen overall in New York state, however, this study has a higher number of participants identifying as Islam.

Table 1. *Demographic information: Age*

	N	Minimum	Maximum	Mean	Std. Deviation
Age	103	17	38	21.97	3.300

Table 2. *Demographic information: Gender, Race, Academic Year, Religion*

		n	%
Gender N = 110	Male	23	20.9
	Female	85	77.3
Race N = 110	Black	34	30.9
	White	34	30.9
	Native American or American Indian	1	0.9
	Hispanic or Latino	17	15.5
	Asian/Pacific Islander	12	10.9
	Other	9	10.9
Academic Year N = 110	Freshman	3	2.7
	Sophomore	14	12.7
	Junior	46	41.8
	Senior	44	40
Religion N = 110	Christian	54	49.1
	Jewish	4	3.6
	Buddhist	2	1.8
	Islam	9	8.2
	Agnostic	1	0.9
	None	17	15.5
	Other	20	7.2

Materials & Procedure

A 33-item survey was constructed from several standardized religious and academic performance assessment surveys. These standardized questionnaires included questions from the Index of Core Spiritual Experiences (INSPIRIT), The Duke University Religion Index (DUREL), the Santa Clara Strength of Religious Faith Questionnaire, and the Approaches and Study Skills Inventory (ASSIST). Questions from the INSPIRIT were used to assess the students' spirituality, DUREL was used to assess religiosity, and ASSIST was used to evaluate

deep, strategic, and surface level study habits. These questionnaires and GPA reportings were used to assess academic performance.

The survey included demographic questions such as age, college level, religious affiliation (if applicable), and race. It also included a religious/spirituality section that assessed whether participants were religious or spiritual with questions such as religiosity level, religious attendance at a place of worship for religiosity, and if they experience feelings of the divine or have a spiritual mentor for spirituality. Academic performance was assessed by asking for participants' GPAs. Spring 2020 participants only were required to place their Fall 2019 cumulative GPA and Fall 2020 students were asked to place both Spring 2020 and Fall 2019 GPAs. Study habits were evaluated by how often they look for deeper meaning in their studies and whether they strategize study materials.

The final section included open opinion questions on religiosity and academic performance such as whether the students felt religion was important to academic success or if success was a possible result of religiosity or spirituality.

All Likert scales items on the constructed survey were on a four-point scale and ranged from strongly disagree to strongly agree, or never to always. Religiosity/spirituality scale participants from 10 (equating to a low religiosity/spirituality) to 40 (equating to a high religiosity/spirituality). A score of 25 suggested moderate religiosity/spirituality. Academic performance was assessed by means of reported grade point averages from both semesters. Scores for study habits also ranged from 10 to 40, with 10 reflecting poor study habits and 40, conversely, indicating better study habits.

The survey was then posted as a link for students to participate. After one to two weeks the survey was closed for the Spring 2020 semester. The same constructed survey was used and

was reopened for Fall 2020 and again closed after one to two weeks. Data was collected and exported from the survey gizmo website and transferred to SPSS Software for appropriate coding and analysis.

Statistical Analysis

To assess descriptive statistics, nominal information is viewed in the frequencies of participant demographic information. Age is a scale measurement with central tendency to view the average age of participants as well as the oldest and youngest. Scale measurement with central tendency also provided average grade point averages for participants. Inferential statistics such as religiosity/spirituality and academic performance, after being transferred to numerical values, are also scale measurements.

RESULTS

Correlation between Religiosity/Spirituality and Academic Performance

To assess the correlation between religiosity/spirituality and academic performance, correlations in SPSS are done through religiosity/spirituality and GPA, as well as religiosity/spirituality and study habits. Mean grade point average for the Fall 2019 semester was 3.22, while the mean grade point average for the Spring 2020 semester was reported at 3.31. Mean scores for religiosity/spirituality were lower than the midpoint of 25 ($M = 21.8774$, $S.D. = 7.78240$), while their mean study habits were above the midpoint of 25 ($M = 26.3030$, $S.D. = 3.71279$).

There were no significant correlations between religiosity/spirituality and academic performance ($dF = 96$, $p = 0.523$, $r = 0.065$). There was also no significant correlation between

religiosity/spirituality and grade point averages for the Fall 2019 semester (dF = 98, p = 0.907, r = -0.012), nor the Spring 2020 semester (dF = 38, p = 0.496, r = -0.109).

Table 3. *Correlation between Religiosity/Spirituality & Academic Performance*

		Religiosity/ Spirituality	Academic Performance
Religiosity/Spirituality	Pearson Correlation	1	0.065
	Sig. (2-tailed)		0.523
	N	106	98
Academic Performance	Pearson Correlation	0.065	1
	Sig. (2-tailed)	0.523	
	N	98	99

Table 4. *Correlation between Religiosity/Spirituality & Grade Point Average*

		Religiosity/ Spirituality	Cumulative GPA as of Fall 2019	Cumulative GPA as of Spring 2020
Religiosity/Spirituality	Pearson Correlation	1	-0.012	-0.109
	Sig. (2-tailed)		0.907	0.496
	N	106	100	41
Cumulative GPA as of Fall 2019	Pearson Correlation	-0.012	1	0.832**
	Sig. (2-tailed)	0.907		0.000
	N	100	101	41
Cumulative GPA as of Spring 2020	Pearson Correlation	-0.109	0.832**	1
	Sig. (2-tailed)	0.496	0.000	
	N	41	41	42

**p < 0 .001

Religiosity/Spirituality and Academic performance by group

In addition to correlational tests, independent groups were assessed in terms of their religiosity/spirituality and their academic performance. Males and females showed no significant results. Males and females had a mean score of 21 and 22, respectively, (p = 0.555, t = 0.592) for religiosity/spirituality. Study habit means also showed no difference in mean with 26 for both

groups (p = 0.374, t = -0.017). Juniors (M = 21.889) and seniors (M = 22.3256) showed no significant values as mean religiosity/spirituality scores also ranged closely (p = 0.797, t = -0.259). Study habit means were 25 for juniors and 36 for seniors (p = 0.781, t = -0.279). Lastly, there were no significant values reported between those who identified as black or African American and those who identified as white.

Table 5. *Group comparison of Religiosity/Spirituality and Academic performance by Gender*

Outcome	Group						95% CI for mean difference	t	df
	Female			Male					
	M	SD	n	M	SD	n			
Religion/ Spirituality	22.1071	7.59045	84	21.0000	8.60786	22	-2.60046, 4.81475	0.592[1]	104
Academic Performance	26.3000	3.54144	80	26.3158	4.47279	19	-1.90605, 1.87447	-0.017[2]	97

1: p > 0.05 (p = 0.555) 2: p > 0 .05 (p = 0.987)

Table 6. *Group comparison of Religiosity/Spirituality and Academic performance by Academic level (Juniors & Seniors)*

Outcome	Group						95% CI for mean difference	t	df
	Juniors			Seniors					
	M	SD	n	M	SD	n			
Religiosity/ Spirituality	21.8889	7.66700	45	22.3256	8.17290	43	-3.79348, 2.92010	- 0.259[1]	86
Academic Performance	25.9762	3.46754	42	26.2051	3.91475	39	-1.86197, 1.40410	-0.279[2]	79

1: p > 0.05 (p = 0 .797) 2: p > (p = 0 .781)

Table 7. *Group comparison of Religiosity/Spirituality and Academic performance by Ethnicity*

Outcome	Group						95% CI for mean difference	t	df
	Black or African American			White					
	M	SD	n	M	SD	n			
Religiosity/ Spirituality	22.7647	7.00242	34	21.7273	8.41974	33	-2.73638, 4.81125	0.549[1]	65
Academic Performance	25.7188	2.95378	32	26.3000	4.83629	30	-2.60268, 1.44018	-0.575[2]	60

1: p > 0.05 (p = 0.585) 2: p > 0.05 (p = 0 .567)

Perceptions of Religiosity/Spirituality and Academic Performance

Participants were asked six questions regarding their opinions of religiosity/spirituality and its impact on academic performance or other aspects of life. When the statement “without my religion or spirituality, I don't think I would do as well academically” was asked, approximately 56% of participants disagreed. Additionally, when the participants were asked the statement “If people were more religious, they would be more likely to succeed,” 64% disagreed.

As for the other four questions similar in fashion, majority of participants agreed with the positive incorporation of religion/spirituality in academia and in life. It can be inferred that the majority of participants agree with the positivity religion/spirituality can have, but disagree with it being necessary for successful personal or school life.

Table 8. *Frequency of "Without my religion or spirituality, I don't think I would do as well academically"*

	Frequency	Percent	Valid Percent	Cumulative Percent
Missing	2	1.8	1.8	1.8
Agree	35	31.8	32.4	34.2
Disagree	34	30.9	31.4	65.6
Strongly Agree	12	10.9	11.1	76.7
Strongly Disagree	27	24.5	25.0	100.0
Total	110	100.0	100.0	

Table 9. *Frequency of "If people were more religious, they would be more likely to succeed"*

	Frequency	Percent	Valid Percent	Cumulative Percent
Missing	2	1.8	1.8	1.8
Agree	24	21.8	22.2	24.0
Disagree	44	40.0	40.7	64.7
Strongly Agree	13	11.8	12.0	76.7

Strongly Disagree	27	24.5	25.0	100.0
Total	110	100.0	100.0	

DISCUSSION

This study sought to determine the correlation between religiosity/spirituality and academic performance through questions of self-reported religiosity and/or spirituality, GPAs, and study habits. It was hypothesized that with higher reports of religiosity and/or spirituality, college students were more likely to have higher GPAs and better study habits resulting in higher academic performance. Much of past literature on this topic supported notions of this hypothesis. However, results indicate retention of null hypothesis.

Although there were no significant findings in the results of this study, demographic information still reports high affiliation with religious denominations or spiritual feelings of connection. This suggests that the foundational teachings of religion or spirituality still may play a critical role in development and education. Group comparisons based on gender, ethnicity, and academic level supplied also had no significant results. Because mean scores for comparable groups were roughly the same, there is no difference among them in terms of their religiosity/spirituality and academic performance. The slight increase in mean scores for black and white students could slightly support Keel's findings, since black or African American students scored slightly lower in academic performance than white students [4].

These findings do not agree with that found by past researchers, as although individuals may identify as religious or spiritual, it does not stand to reason that they will perform better academically or have better study habits. Participants' disagreement with the statement that religiosity/spirituality was necessary for academic success, supports the aforementioned idea. This could also suggest that students farther in their path of higher learning are not heavily

influenced by their religiosity/spirituality to the extent that there is no relationship between their religiosity/spirituality and their ability to succeed academically. Another possibility is that no matter how religious or spiritual someone purports to be, it does not guarantee academic success.

The results of the group comparison, however, do agree with the spirituality and religiosity levels of Luquis, Brelsford, and Rojas-Guyler's findings with respect to males and females [5]. In this study, The Brief Multidimensional Measurement of Religiousness and Spirituality (BMMRS) was used in the study's questionnaire to measure their respective sample's religiosity and spirituality. The researchers also used the Daily Spiritual Experiences (DSES) scale, along with the Private Religious Practices (PRP) scale. These results with population statistics instill confidence that the results of this study are reflective of how religiosity and spirituality are practiced respective to gender in college students.

Academic performance in college juniors and seniors would also support DeMoulin's findings, as academic performance means were somewhat higher in this study's sample of seniors than in juniors [1]. The comparative sample within this study had group samples that were better represented, yet still provided the parallel results. Academic performance was defined by study habit factors rather than GPAs alone for the purpose of assessing the target variables; however, it can be argued that DeMoulin's results are still an important indicator of the slight success differences between the two aforementioned groups [1].

From the correlational results, it can be hypothesized that the effect of religiosity/spirituality and academic performance are limited to primary and secondary learning (elementary school, intermediate school, and high school). Lee et al., study was conducted on grade level students producing positive correlations, while Good and Willoughby conducted the same study among adolescents [6,2].

Some limitations of this study include inclusion criteria of New York State College students and would have more potential to generalize if several students from other educational organizations were included within the study. It would also benefit students to further research these correlations with all levels of education. Future researchers may benefit in this area by including a larger number of participants who may not affiliate with any religious or spiritual foundation (atheist) and compare the correlational values between students who are/are not religious to determine if there is any significance to the academic performance of atheistic students.

Some highlights include a sufficiently constructed survey to gather information about religiosity and the longitudinal study style, which allowed for additional participants to assess for any potential significance that may have appeared in larger sample sizes. It is also important to note the difference in opinions about religiosity/spirituality and their importance in students' lives as they perceive. This could suggest that although the majority of students in college may identify with religiosity and/or spirituality, there is no belief that it is the reason attributed to their success.

CONCLUSION

Past literature indicates positive correlations between students' academic performance and religiosity/spirituality. Other variables have also been shown to aid or hurt students' ability to succeed in school (i.e. stress, nutrition, sleep, caffeine etc.). As college students, educators and students would benefit from knowing what is required to do well in school and grow through life and its events. It should also be taken into account that everyone is different, and what may help advance one student may not be effective for others. Therefore, it is important to find and test all potential variables, so students can mold themselves according to their specific preference.

Findings such as these also help colleges and educational institutions help their students achieve success by fostering these different variables that result in better grades, which many do. Activities such as yoga nights to meditation sessions, or art workshops can help to calm anxious students, or simply allow for social interaction between peers. As students and future researchers, our work to better ourselves and the incoming generations is valuable, and sets the stage for new, accurate, and applicable knowledge.

It should also be noted within the open discussion of religiosity/spirituality and its effects on students, that although the most recent studies in this area were conducted with grade school students, there could be a further connection between education level and how much of an influence religion has on academic success. It could further the understanding between the connection of those who identify as atheists and their majority education level versus those who identify as religious/spiritual and their majority educational level. A study conducted by M Stirrat and RE Cornwell displayed that scientists (particularly biological scientists) are far more unlikely to believe in the existence of god, hold beliefs in a personal god, or believe in continued consciousness or life after death [10]. Paralleled with no significant correlation being found among religiosity/spirituality and academic performance, one can imply that a further study could potentially indicate a negative correlation between religiosity and higher learning (particularly in the sciences) as well as a lack of necessity for religiosity to succeed in higher learning.

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Covid-19 and its Effect on Stress Levels and Academic Performance in College Students

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ABSTRACT

The present study explored the relationships between the presence of Covid-19 and how it affects stress and academic performance in college students. Participants (n = 80) were asked to complete a survey regarding their experience with Covid-19 as well as their stress levels and academic performance during the Spring 2020 semester. Results for Research Question 1 (r (79) = 0.568 p = 0.000) showed that there was a positive correlation between Covid-19 and increased stress levels. Results for Research Question 2 (r (79) = 0.584 p = 0.000) also presented a strong, statistical correlation between Covid-19 and academic performance. Although students reported high worries over Covid-19, most students surprisingly performed higher than average. It is suggested that further research should be done in order to determine if other factors may be involved in determining why GPA (Grade Point Average) and stress levels were affected in conjunction with Covid.

INTRODUCTION/BACKGROUND

The novel coronavirus is one of the many diseases that have infiltrated and impacted people worldwide. Coronavirus-19 (Covid-19) originated as an outbreak in China in late 2019 and has been declared a global pandemic since March 2020. This pandemic has not only affected the physical health of the global population but has also affected mental health and stress levels.

College students not only have to focus on their academic careers but also have to undergo extreme, extenuating circumstances that come with the novel coronavirus. Increased fear of family, friends, or themselves contracting the virus, as well as keeping up with home quarantine and remote learning, could potentially lead to an increased level of stress. This increase in stress could affect their academic performance. Therefore, this study explored how the global pandemic (Covid-19) affects stress levels and academic performance in college students.

In a world where Covid-19 is still new and constantly evolving, it is important to conduct studies that determine the implications that may occur and how college students may be affected. This study asks the following research questions:

RQ_1: Does the presence of Covid-19 have an effect on stress levels in college students?

H_1: The presence of Covid-19 has a negative effect on stress levels in college students.

RQ_2: Does the presence of Covid-19 have an effect on academic performance in college students?

H_2: The presence of Covid-19 has a negative effect on academic performance in college students.

LITERATURE REVIEW

What is a Pandemic and COVID-19?

According to the World Health Organization, a pandemic is defined as "the worldwide spread of a new disease" [12]. Globally, there have been many health challenges due to the virus that has affected millions of people. While Covid-19 is known to exhibit pneumonia-like symptoms in the body, another threat has also developed: psychological distress. Historically, there is evidence that pandemics, outbreaks, as well as other global crises, led to psychological trauma in individuals. With Covid-19 specifically, this trauma has only intensified due to the ever-changing healthcare environment that surrounds the disease.

COVID-19 and its Impact on Mental Health

The Covid-19 pandemic has multiple impacts on mental health. Many young adults may have pre-existing mental health conditions that have only been exacerbated since the start of the pandemic. With the growing concern of contracting the disease, public and state officials have mandated "stay-at-home orders" for the general public. Individuals that are more prone to mental health issues could possibly be affected by Covid-19 at a greater degree in comparison to those who do not have pre-existing mental health issues [1]. Although young adults have a lower risk of contracting the coronavirus, there is still an undeniable increased risk of stress-related health issues.

What is Stress?

According to the American Institute of Stress, stress is defined as "the non-specific response of the body to any demand for change" [13]. Stress affects everyone at some point in their lives, some more so than others. In relation to the Covid-19 pandemic, stress can be attributed to factors such as the lack of a cure or vaccine available, loneliness due to home quarantine, and

financial instability due to job loss caused by a diminished pandemic economy. Individuals who have pre-existing mental health issues may show an increased risk of having new stress-related disorders directly due to the coronavirus [6]. Although the virus has a relatively low mortality rate, there is still an increased impact on mental health.

According to Shapiro, Levine & Kay (2020), a sample of 503 participants (61% female, 39% male) based in Israel completed a questionnaire that evaluated their mental health and stressors during the ongoing Covid-19 pandemic [9]. Researchers found that 12% of their original sample were at risk of depression. Specific stressors the respondents recorded include being worried about family and friends contracting the virus, their own economic and financial situations, isolation and loneliness, and interrupted/restricted daily life [9]. Approximately 75% of participants reported having high or very high stress levels during this time. However, only 21% of respondents sought to seek professional help. Due to the presence of Covid-19, stress levels increased exponentially.

Covid-19 and Stress

As Covid-19 has spread worldwide the virus has evidently impacted people's physical and mental well-being, most notably their stress levels. Qiu et al. (2020) found young adults had higher scores in relation to increased stress levels due to prominent information from news sources as well as social networking sites [8]. With more information available revolving around the risk and health implications of the virus, many people are understandably worried about their health. Interestingly, individuals with higher education also reported increased stress in relation to the presence of Covid-19.

Stress Among College Students

According to Garrett, Liu & Young (2017), 75% of undergraduate college students have experienced at least a moderate amount of stress [5]. Some stressors for college students include pressure for high academic performance, personal relationships, and poor coping skills. Based on research done by Garrett et al. (2017), a sample of 197 college students participated in a longitudinal study where they completed questionnaires pertaining to stress levels during their freshman year [5]. Results (based on a Likert scale of 1 = minimal stress through 5 = extreme stress) showed an average stress level of 3.4 (SD = 0.99), which presented a higher-than-average stress level. Results also showed peaks during midterm exams (M = 3.57), as well as final examination week (M = 3.95). With higher stress levels, there are associations with lower sleep quality, lower emotions of love and joy, and increased emotions of fear and anger [5]. Stress can also have an effect on academic performance in college students.

As a college student, there is an underlying pressure to perform well academically. Having high levels of stress may impact academic performance in a negative way. According to Lee, Kang & Yum (2005), students attending a large university in Korea reported the number one academic stressor to be grades and competition [7]. Vaez and Laflamme (2008) found students who experienced one of their 14 indicators of stress had a decreased GPA which in turn, affected their ability to obtain their degree [11]. Stress due to "not coping academically" as well as "study support demands" affected students' performance significantly. The added stressors of a global pandemic on college students have the risk of affecting GPA and academic performance even more.

Covid-19 and Academic Performance

Due to the pandemic, there are many stressors that college students may encounter. One stressor may be how Covid-19 could affect academic performance in college students. Cao et al.

(2020) found that college students based at a university in China experienced anxiety due to the Covid-19 outbreak [3]. This anxiety could potentially be attributed to how Covid-19 had impacted their academic performance.

METHOD

Design

The present study used a correlational research design study that utilized methods that examined the relationship between the presence of a global pandemic (Covid-19) and how it affects stress levels as well as academic performance in college students. The variables in this study include Covid-19, stress levels, and academic performance. Data from this study was collected through an online survey (SurveyGizmo) from participants who self-reported their responses.

Participants

The present study included 80 participants (85% female, 13.8% male, 1.3% Other; M_{Age}= 22.81 years, S.D. = 4.91) who attended college or university during the Spring 2020 semester. Students self-identified as multiple ethnic backgrounds, including Asian or Pacific Islander (18.8%), Hispanic or Latino (22.5%), White (41.3%), and Black or African American (17.5%). Of the 80 participants, 88.8% reported they commuted to campus, while 11.3% said they lived on campus. Participation in this study was on a voluntary basis and those who participated were not compensated for their involvement.

Table 1

Study sample age characteristics (n = 80)

Age

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age	80	17	45	22.81	4.910
Valid N (listwise)	80				

Table 2

Study sample ethnicity characteristics (n = 80)

Ethnicity

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Asian or Pacific Islander	15	18.8	18.8	18.8
	Black or African American	14	17.5	17.5	36.3
	Hispanic or Latino	18	22.5	22.5	58.8
	White	33	41.3	41.3	100.0
	Total	80	100.0	100.0	

LivingSituation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	I live alone	3	3.8	3.8	3.8
	I live with family	65	81.3	81.3	85.0
	I live with friends/roommates	4	5.0	5.0	90.0
	I live with my family	1	1.3	1.3	91.3
	I live with my significant other	7	8.8	8.8	100.0
	Total	80	100.0	100.0	

Table 3

Study sample living situation characteristics (n = 80)

Materials and Procedures

The present study (n = 80) utilized a self-report survey, which was hosted by SurveyGizmo.com through a link to collect participants' data. The survey included questions that asked participants about their feelings and experiences with the global pandemic (Covid-19) and their stress levels and academic performance during the Spring 2020 semester. Questions pertaining to Covid-19 were modeled based on the COVID Stress Scale by Taylor et al. (2020) [10]. Stress levels were measured through the online survey and utilized questions from the Perceived Stress Scale (PSS) by Cohen [4]. Academic performance was measured by questions utilizing the Approaches and Study Skills Inventory for Students (ASSIST) by Brown et al. (2015) [2].

The present study survey consisted of 55 questions separated into five sections. Participants responded to background questions regarding their age, gender, ethnicity, and class standing. Section I pertained to demographic information (age, gender, commute vs. live on campus, etc.). Section II was related to their experience with Covid-19. Section III asked questions based on their stress levels within the past three months. Section IV pertained to their academic performance during the Spring 2020 semester. Lastly, Section V asked participants for their opinion on how Covid-19 has affected their academic performance during the Spring 2020 semester. The survey included questions such as, "What is your current living condition?", "In the last month, how often have you felt that you were unable to control the important things in your life?", and "What was your semester GPA prior to the Spring 2020 semester?" 32 of the 42 questions utilized responses that consisted of a five-point Likert scale.

Participants were tested during their free time and after giving their consent, they received written instructions at the start of the survey. Participants were given an unlimited amount of time to complete their responses. After submitting their replies, they were thanked for their time

and contribution to the survey. Once the survey was completed and all answers were accounted for, each section was put into Excel to "clean" the data. Once the data was cleaned, it was uploaded into SPSS Software for further analysis.

Statistical Analyses

The study utilized Pearson Correlation to compare two variables per hypothesis. For H_1, variable 1 was Covid-19 and variable 2 was stress levels. For H_2, variable 1 was Covid-19 and variable 2 was academic performance. The rejection level for all data was $p < 0.05$.

For Section II (Your experience with Covid-19), scores ranged from 0 - 40. The midpoint was 20, and therefore, higher scores equal more worry about Covid-19. For Section III (Stress Levels), scores ranged from 0 - 40. The midpoint was 20, and therefore, higher scores equal higher stress levels. For Section IV (Academic Performance), scores ranged from 0 - 40. The midpoint was 20, and therefore, higher scores equal better academic performance. Finally, for Section V (How Covid-19 has affected your academic performance), scores ranged from 0-16. The midpoint was 8, and therefore, higher scores equal more worry about Covid-19 affecting academic performance.

RESULTS

RQ1: Does the presence of Covid-19 have an effect on stress levels in college students?

To address this question, Pearson's correlation was used to determine the relationship between the presence and experience of a global pandemic (Covid-19) ($M = 25.63$, $S.D. = 9.723$) and stress levels ($M = 22.82$, $S.D. = 7.045$) in college students. The results ($r(47) = 0.594$ $p = 0.000$) revealed that there is a strong correlation. In the sample, participants reported an increase in stress levels due to their experience and overall opinions of Covid-19 as shown in **Figure 1**.

The present study supports the original hypothesis that states the presence of a global pandemic (Covid-19) has a negative effect on stress levels in college students, therefore, the null hypothesis is rejected. The correlation was statistically significant and had a strong, positive correlation between the two variables.

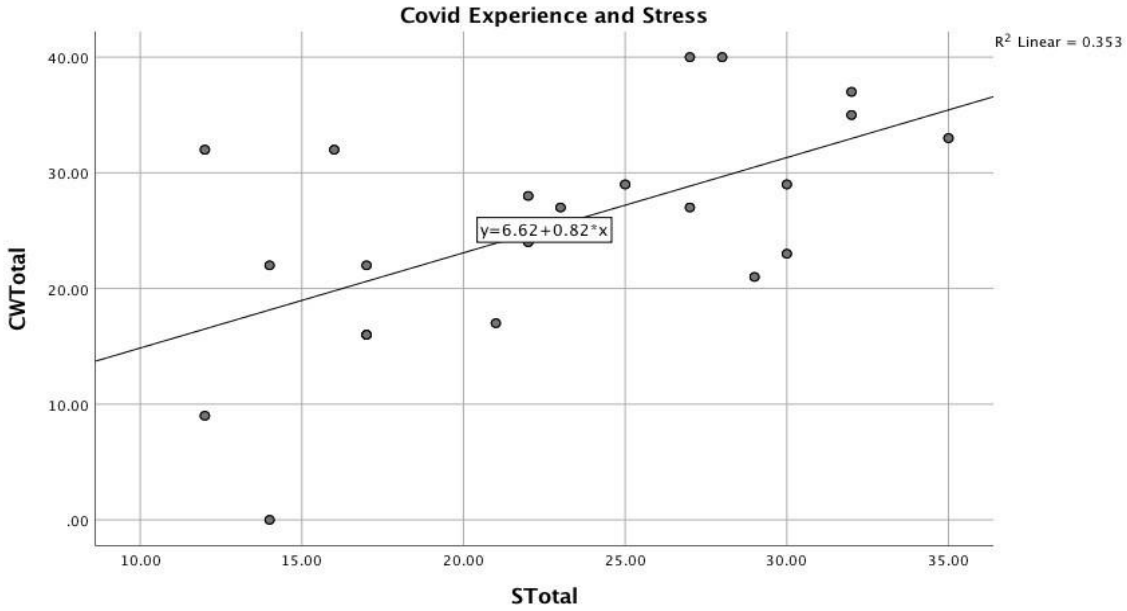

Figure 1. Scatter plot of Covid-19 experience and stress levels.

Table 4

Descriptive Statistics of Covid Experience and Stress Levels

	N	Minimum	Maximum	Mean	Std. Deviation
Covid Experience	48	.00	40.00	25.6250	9.72303
Stress Levels	44	12.00	35.00	22.8182	7.04561

RQ2: Does the presence of Covid-19 have an effect on academic performance in college students?

To address this question, Pearson's correlation was used to determine the relationship between the presence of a global pandemic (Covid-19) (M = 25.63, S.D. = 9.723) and academic performance (M = 24.57, S.D. = 5.749) in college students. The results (r (47) = 0.652 p = 0.000) revealed that there is a strong and positive correlation between the two variables. Although participants reported an increased worry due to the presence of a global pandemic (Covid-19), students reported either average or above-average GPA and moderate to high academic performance during their Spring 2020 semester as observed in **Figure 2**. The correlation was statistically significant and had a strong, positive correlation between the two variables. The original hypothesis that stated the presence of a global pandemic (Covid-19) has a negative effect on academic performance is not supported by this study.

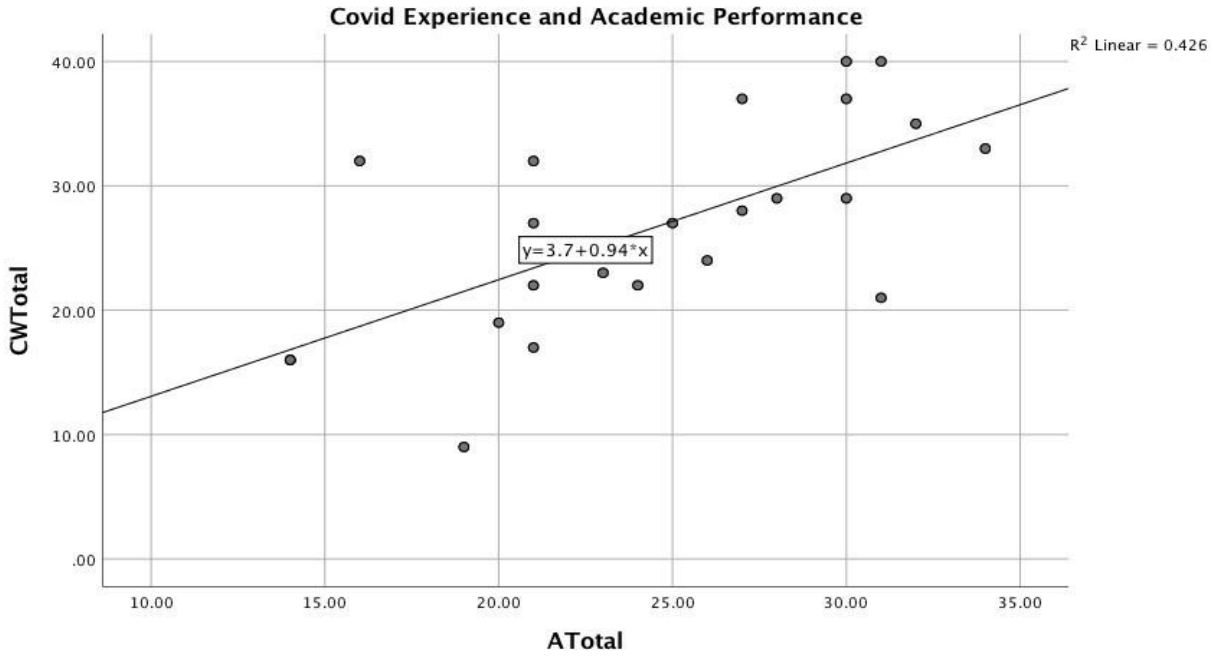

Figure 2. Scatter plot of Covid-19 experience and academic performance.

Table 5

Descriptive Statistics of Covid Experience and Academic Performance

	N	Minimum	Maximum	Mean	Std. Deviation
Covid-19 Worries	48	.00	40.00	25.6250	9.72303
Academic Performance	46	14.00	34.00	24.5652	5.74902

DISCUSSION

The present study attempted to examine the relationship between the presence of a global pandemic (Covid-19) and how it affected stress levels and academic performance in college students. The main purpose of this study was to determine whether Covid-19 had negatively affected stress and academic performance. As shown in the results, there is a significant correlation found between Covid-19 and stress levels as well as statistical significance between Covid-19 and academic performance. Results for research question 2, specifically, were the most surprising. Despite the widespread of Covid-19 and its consequential spread of panic, the majority of college students who were sampled showed either average or above-average academic performance during the Spring 2020 semester.

The results for the participants' experience with Covid-19 and stress levels are consistent with data done by Qiu et al. (2020) [8]. As the concern for Covid-19 increased, so did student's stress levels. This unexpectedly resulted in an averaged or increased student academic performance.

For Group 2, although there was significance between stress and academic performance, students may have been able to persevere and handle their academic responsibilities despite their stress levels. Stress levels increased significantly; however, students still managed to score average to high GPAs. There was little to no correlation between Covid-19 and academic performance and this may be due to the "normality" of exposure. Months after the outbreak, students were subjected to online learning and by the Fall 2020 semester began, students were able to navigate virtual academia more confidently than their previous semester; this could be the reason for less worry over Covid-19 affecting their academic performance.

Study Limitations

This study's results indicate that there is a positive correlation between Covid-19 and an increase in stress levels. There was also statistical significance between Covid-19 and academic performance. One potential limitation may be the sample size. The study's sample may not provide a true representation of how college students experience Covid-19, and may not portray accurate stress levels and academic performance. In order to achieve a more accurate result, a larger sample size should be implemented. Another limitation is the utilization of self-reported surveys. Some participants, for example, may not have been completely honest or conversely, felt as though they needed to exaggerate or inflate their answers such as GPA Also, many universities switched to remote learning, meaning all classes moved to an online setting. Therefore, the survey results may not have represented inflated grades due to online/remote work, which could have skewed results favorably in terms of GPA.

Implications for Future Research

The study's results showed the effects of a global pandemic on college students. Due to the limited sample size, further testing and studies on Covid-19's effects should be done in larger populations. One thing that was interesting to note was that participants were mostly recruited from American cities, according to SurveyGizmo analytics. Possible addition for further research could be the testing of college students in other countries that have also been affected by Covid-19 as there may be cultural differences that could change or skew results. A second addition could be the testing of young, school-aged children in order to determine whether Covid-19 also affects their stress levels and academic performance.

CONCLUSION

The findings of this study indicate that Covid-19 has an impact on stress levels and academic performance in college students. With the presence of the pandemic continuously and rapidly changing, Covid-19's lasting impact may not only be physically draining but mentally as well. It is important to note that this study's findings generalize the impact that the presence of a global pandemic may have on college students' stress levels and academic performance. The presence of Covid-19 and its impact suggests college, government, and mental health sources work collaboratively to resolve heightened panic, worry, and stress levels in college students. This research was important in determining the mental health and academic resources that can be made available for college students in the event of a global pandemic.

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Synthesis of Two New Crown-Ether Cyclophanes for Measuring Cation-Aromatic Interactions in Solution

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ABSTRACT

Molecular recognition is the specific noncovalent interaction between two or more atoms or molecules, such as hydrogen bonding, metal coordination, and hydrophobic forces. It plays a role in biological systems as well as in the development of new technologies. Consequently, molecular recognition is crucial for us to study and become more familiar with these interactions. In this study, we have synthesized two new 15-crown-5 ether cyclophanes: compounds **1** and **2**. Compound **1** consists of a phenyl ring as the spacer, while compound **2** possesses an anthracenyl spacer. Using the cyclophanes as *host* molecules, we performed molecular-recognition studies using potassium ion and sodium ion as the *guest* cations. With known concentrations of each host and using proton NMR spectroscopy, we performed a titration experiment with known aliquots of potassium hexafluorophosphate and sodium hexafluorophosphate in acetonitrile separately. Our goals are to determine the association constants of each host compound with the guest cations and ascertain the contribution of cation-π interaction in the molecular recognition process by comparing the association constants of the two cyclophane hosts. Noting that the structural difference between the two host compounds relates to the π electron density of anthracene being superior to that of the benzene, the difference in the association constants was used as a measure of the cation-π interaction. With the aid of the *Bindfit* program, the changes in the proton NMR chemical shift as a function of concentration enabled us to determine an association constant of 24.60 M^{-1} for host 1 with K^+ and an association constant of 49.98 M^{-1} for

host 2 with K^+, which amounted to association energies of -1.897 kcal/mol for host 1 and -2.316 kcal/mol for the host 2. With Na^+, we determined the association constant for host 1 to be 11.24 M^{-1} and for host 2 to be 7.95 M^{-1}.

INTRODUCTION

Crown ethers are cyclic molecules made up of hydrogen, carbon, and oxygen, where each oxygen atom is bound between two carbon atoms (ether functional group) [2]. They are called 'crown' ethers because their three-dimensional shape resembles that of a crown. Some examples of crown ethers are included in **Figure 1**.

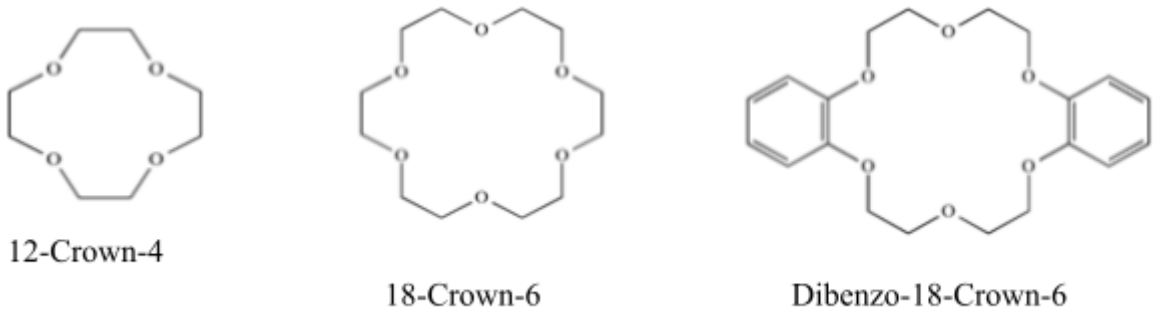

Figure 1. Examples of crown ethers.

Additionally, cyclophanes are cyclic compounds containing at least one aromatic ring in the closure of the larger ring [3]. Crown ethers are remarkable for their ability to exhibit selective cation binding [5]. Atoms like sodium and potassium, which contain specific metallic properties, pass through the center of the ring and are able to attach themselves to the exposed oxygen atoms. They fit somewhat like a key in a lock, depending on the element as well as the size of the ring. The crown molecule can act as a "host," and it is able to take its "guest" to a place where it would not go on its own [5]. For example, a crown ether can take a cation through the membrane

that makes up the wall of a cell. Crown ethers have high selectivity which allows them to "identify" the guest atom in a solution and effectively wrap around it.

Crown ethers are able to mimic, in a not-so-complicated way, the vastly complicated functions of biological systems, enzymes for instance [5]. This mimicry has made them fascinating molecules for various fields and their study could result in the development of new pharmaceutical systems. Crown ethers could potentially serve as a natural method of crossing the blood-brain barrier and could help us understand how the body moves essential elements like sodium and potassium into cells [5]. Other types of uses for crown ethers include their potential to serve as "scavengers," where they would be able to remove elements like radioactive strontium from the environment [5]. This property would allow them to also regulate concentrations of sodium in the blood. Researchers have even suggested that crown ethers could someday be used to extract uranium or gold from seawater [5].

Scientists have been studying the cation-aromatic interaction in various crown-ethers or cyclophanes with cations. The most relevant example to our study comes from the experimentation and analysis done with the complexation of metal ions with chromogenic Calix[4]biscrowns. This was completed with the use of UV/VIS spectroscopy and the ENZFITTER program [4].

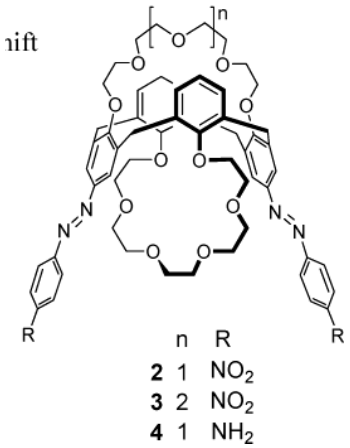

Figure 2. Azo-coupled calix[4]biscrown compounds.

A series of 1,3-alternate chromogenic azo-coupled calix[4]biscrowns had been synthesized, as pictured in **Figure 2**. It is seen that the molecules differed in functional groups denoted by R and/or the crown size was varied with crown-5 and crown-6 (denoted by n). The scientists' research studied how changing the functional groups or structure of the molecule impacted the amount of interaction between the chromogenic Calix[4]biscrown molecule and the metal ion. They also measured the amount of interaction each metal ion had with the chromogenic Calix[4]biscrown molecules through computing association constants for the complexes. This is very similar to our experiment as we also observe how the aromatic-cation interaction changes with structural differences and observe and measure this interaction through evaluating association constants.

In the experiments the scientists had conducted, they used various metal ions. A host-guest titration was performed, and from the results of UV/vis band shift upon metal ion complexation, metal ions were entrapped only by the upper crown loop, causing the hypsochromic shift on the UV/vis spectra.

compd	property	Li^+	Na^+	K^+	Rb^+	Cs^+	Ca^{2+}
2	K_a (10^3)	0.85	2.46	822.1	652.5	48.5	1.72
	$-\Delta G^b$	16.7	19.3	33.7	33.1	26.7	18.4
3	K_a (10^3)	1.20	0.98	5.86	6.58	223.6	2.41
	$-\Delta G^b$	17.5	17.0	21.5	21.7	30.5	19.3

[a] The K_a (M^{-1}) values were obtained from the ENZFITTER program. [b] kJ/mol.

Figure 3. Association constants (K_a)[a] and free energy of compounds 2 and 3 for metal cations.

Compound 2 revealed K^+ ion selectivity, while compound 3 showed Cs^+ ion selectivity caused by a size complementarity between hosts and guest ions. From the UV band shift of compound 4 in which the NO_2 group was replaced by the NH_2 group, they observed bathochromic shift upon the metal ion addition, indicating that the metal ion is encapsulated in the lower crown ring because of strengthened π -cation interaction by introducing the electron-donating NH_2 unit regardless of the steric hindrance between two azo-phenyl groups adjacent to the crown ring [4].

In this experiment, two host molecules were utilized: host 1 and host 2. Their chemical structures are shown below in **Figure 4**.

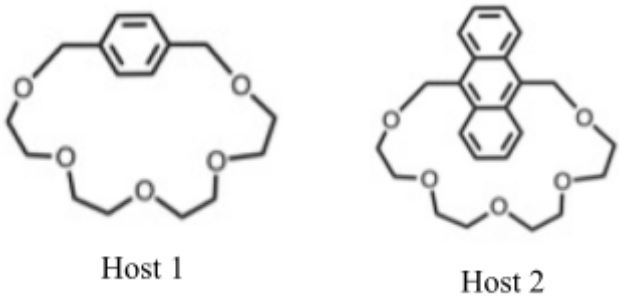

Figure 4. Two newly synthesized crown-ether cyclophanes.

The objective was to insert the guest, a potassium or sodium cation, into the cavity of the crown ether and calculate the association constants of the resulting complex. This could be done using NMR Spectroscopy and the *BindFit* program, which has been used before for

equilibrium/association constant quantification. The association constant is highly dependent on the interactions that are occurring between the host and guest molecules; in this case, the interactions occur between the cation and oxygen atoms and between the cation and the π-electrons in the aromatic rings. The cations were obtained from potassium hexafluorophosphate and sodium hexafluorophosphate; their molecular structures are shown in **Figure 5**.

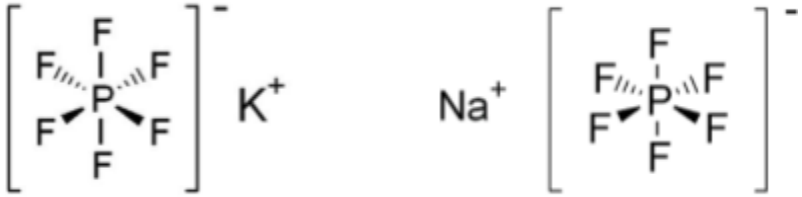

Figure 5. Two-dimensional molecular structures of potassium hexafluorophosphate and sodium hexafluorophosphate.

When potassium hexafluorophosphate is dissolved in a polar solvent, the K^+ will separate from the negatively charged hexafluorophosphate, and the same separation occurs in sodium hexafluorophosphate when dissolving in a polar solvent. These cations are now able to move freely in the solution, and thus, can bind in the cavity of the crown ethers and also have an interaction with the aromatic rings.

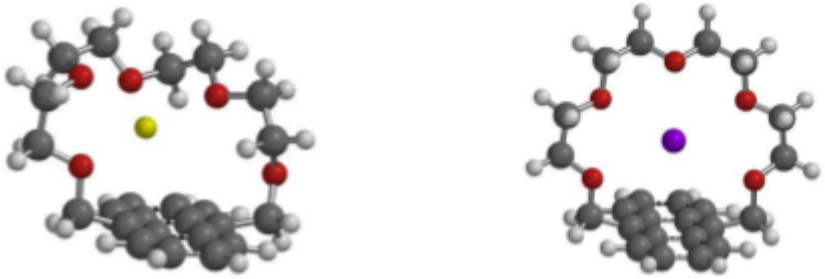

Figure 6. Three-dimensional models of two of the host-guest complexes; the Host 2-Na^+ complex is pictured on the left, and the Host 2-K^+ complex is pictured on the right.

PROCEDURES AND METHODS

The experiment was initiated by obtaining two clean vials and 10-uL, 25-uL, and 500-uL syringes. Thereafter, around 15 mg of host 1 was measured and added to one of the vials. To follow, host 2 was required to have the equivalent number of moles as host 1, so the mass of host 2 that provided the same number of moles as the sample of host 1 was calculated, measured, and added to the same vial. To dissolve the two host compounds, 620 uL of deuterated acetonitrile was added to the vial, and the vial was shaken. Subsequently, the molarity of each host and the total molarity of the solution were calculated; the guest compound (the potassium hexafluorophosphate) was chosen to have a concentration that is approximately 24 times more than the total concentration of the hosts' solution. With the guest concentration and the selected volume of deuterated acetonitrile – 655 uL – the mass of potassium hexafluorophosphate needed to create this concentration was calculated, measured, and added to a second vial. The 655 uL of deuterated acetonitrile was measured afterward and added to the same vial. The vial was shaken until the guest was fully dissolved. After, the solution containing the hosts was transferred into an NMR tube, and the first NMR reading of the hosts alone (without any of the guests) was taken. The first addition of the guest was made with a chosen volume, the NMR tube was shaken well, and the concentration of the guest added was calculated. An NMR reading was taken after each guest addition, and the NMR spectrum of this was compared to the NMR spectrum of the hosts' solution with no guest addition. Afterward, more additions of the guest and more NMR readings were taken until there were no significant changes observed in the peaks. Thereafter, one signal for host 1 and one signal for host 2 was chosen to be examined; the chemical shifts at the beginning of the titration experiment and the change in chemical shifts after each addition were recorded for both of the chosen signals. Following this, the host concentration, the changes

in guest concentration, and the changes in chemical shift were imputed into an Excel sheet, separately for each host. The excel sheets were independently uploaded to the *BindFit* program, and the association constant and a plot was obtained for each host. Lastly, the association energy of the complex was calculated for each host, and the difference of the association energy was computed as well. After these steps were completed for the K^+ guest, the same was followed for the Na^+ guest, but the association energy calculation was excluded.

RESULTS

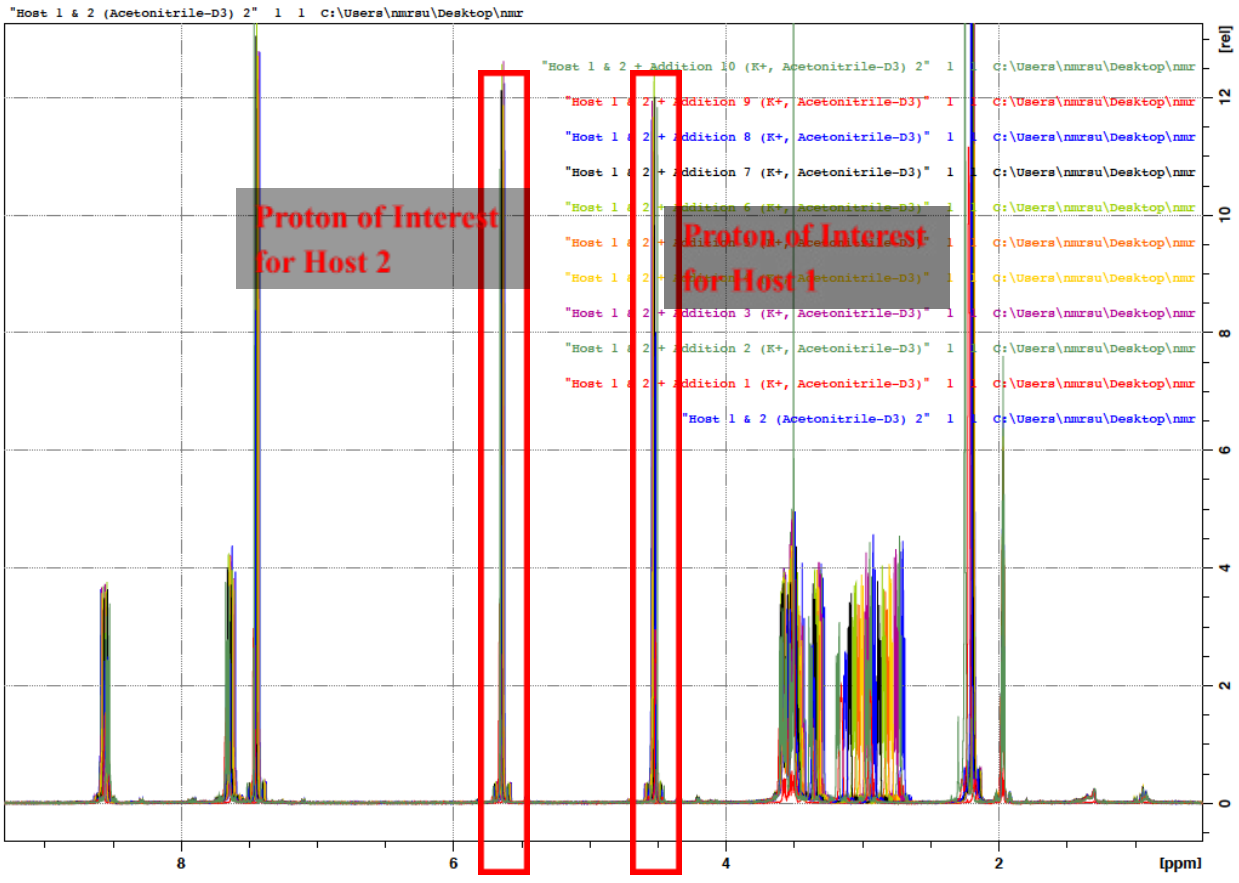

Figure 7. NMR spectrums of host 1, host 2, and guest additions (as K^+ as the guest

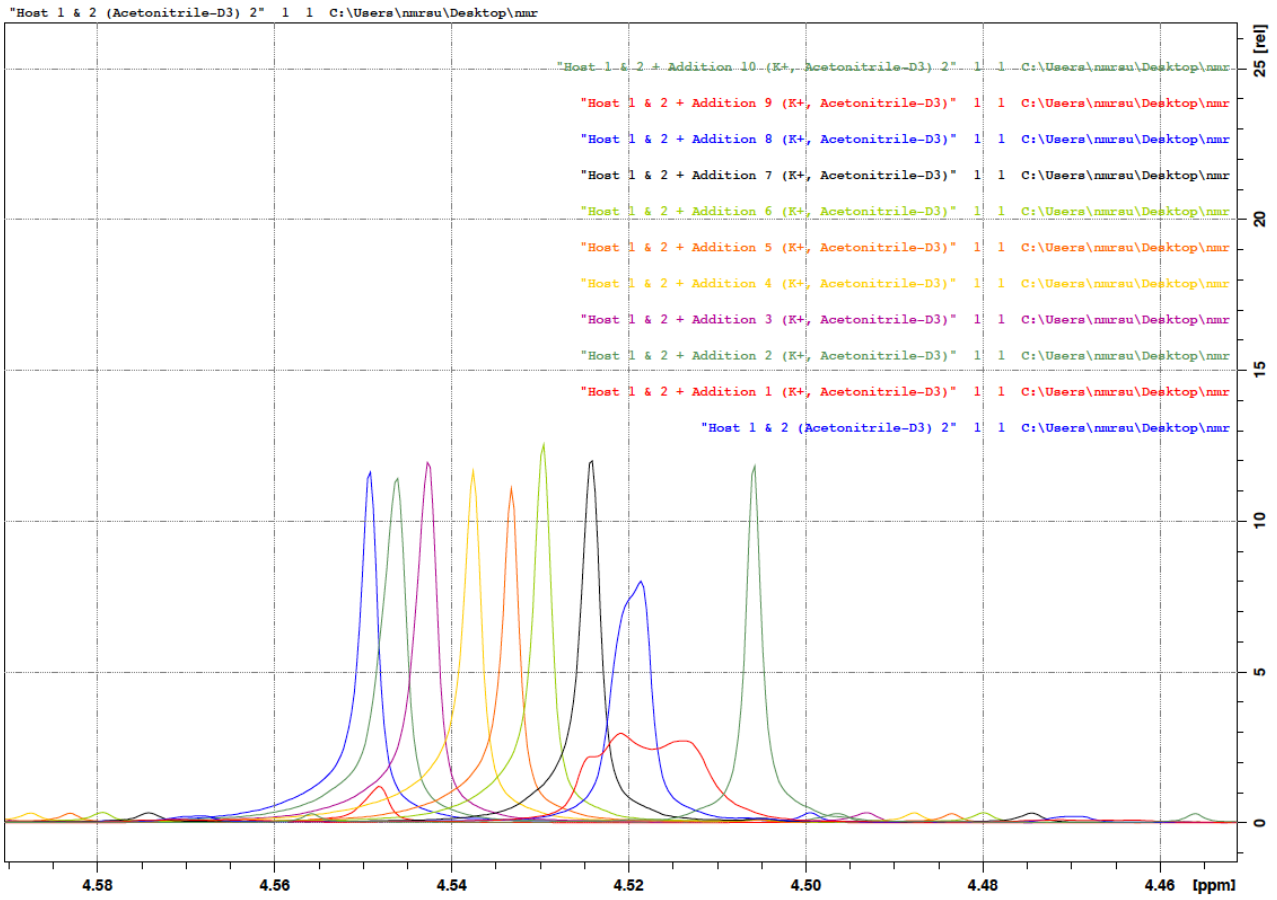

Figure 8. Proton of interest for host 1 with K^+ as the guest

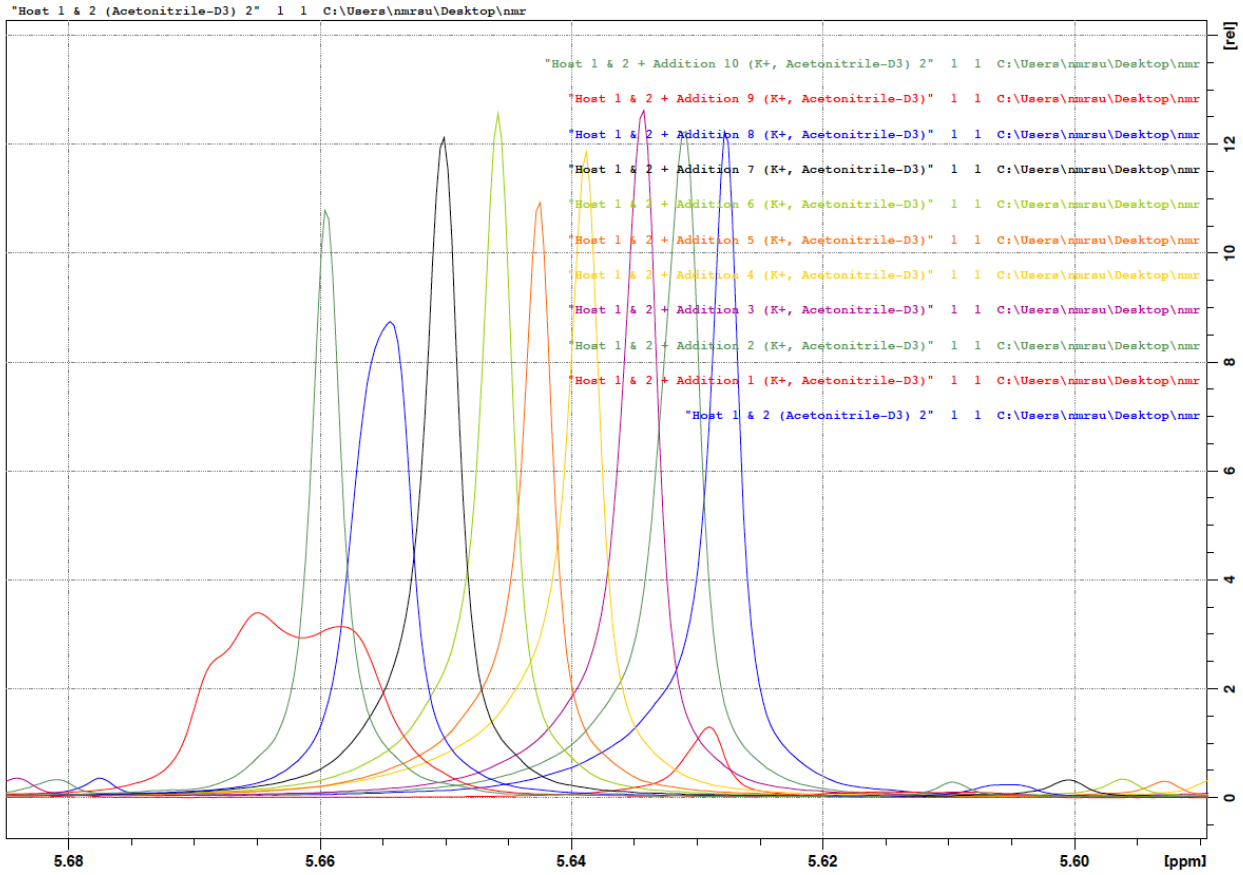

Figure 9. Proton of interest for host 2 with K^+ as the guest.

***Note (for both Figures 8 and 9)**: it was discovered that re-shimming was required prior to taking the NMR reading for the ninth addition. This was not done, so the "Host 1 & 2 Addition 9 (K^+, Acetonitrile)" peak came out indecisive. Since at least eight data points for the additions are required, this peak was disregarded, and nine of the remaining data points for the additions were used; this did not affect the data.

Host 1 Concentration (M)	Addition of Guest	Guest Concentration (M)	Chemical Shift (ppm)
0.01360610		0	4.5493
0.01360610	Addition 1: 1.0	0.00106721	4.5482
0.01360610	Addition 2: 2.0	0.00319134	4.5462
0.01360610	Addition 3: 5.0	0.00842425	4.5427
0.01360610	Addition 4: 5.0	0.01361068	4.5376
0.01360610	Addition 5: 5.0	0.01869786	4.5333
0.01360610	Addition 6: 5.0	0.02370593	4.5297
0.01360610	Addition 7: 10.0	0.03349192	4.5243
0.01360610	Addition 8: 15.0	0.04762170	4.5197
0.01360610	Addition 9: 95.0	0.12420860	4.5059

Table 1. For host 1, host concentration, additions of guest, changes in guest concentration & changes in chemical shift with K^+ as the guest.

Host 2 Concentration (M)	Addition of Guest (uL)	Guest Concentration (M)	Chemical Shift (ppm)
0.01360610		0	5.6277
0.01360610	Addition 1: 1.0	0.00106721	5.6291
0.01360610	Addition 2: 2.0	0.00319134	5.6310
0.01360610	Addition 3: 5.0	0.00842425	5.6344
0.01360610	Addition 4: 5.0	0.01361068	5.6389
0.01360610	Addition 5: 5.0	0.01869786	5.6426
0.01360610	Addition 6: 5.0	0.02370593	5.6459

0.01360610	Addition 7: 10.0	0.03349192	5.6502
0.01360610	Addition 8: 15.0	0.04762170	5.6548
0.01360610	Addition 9: 95.0	0.12420860	5.6595

Table 2. For host 2, host concentration, additions of guest, changes in guest concentration & changes in chemical shift with K^+ as the guest.

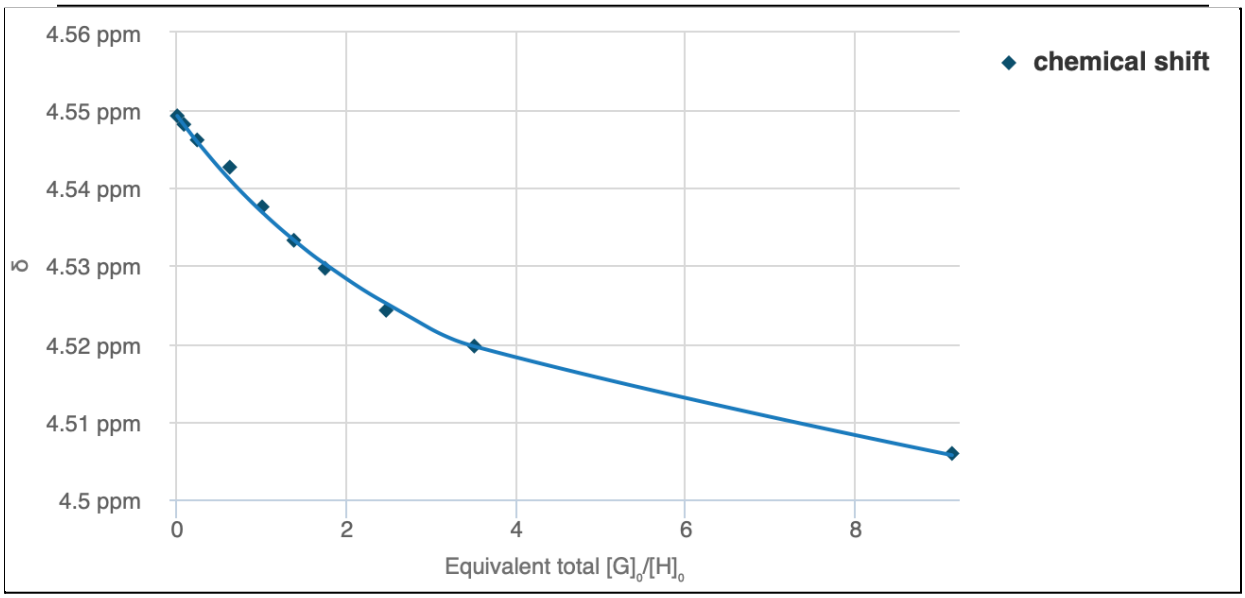

Figure 10. The fit of chemical shift (ppm) against guest concentration (M) over host 1 concentration (M) with K^+ as the guest.

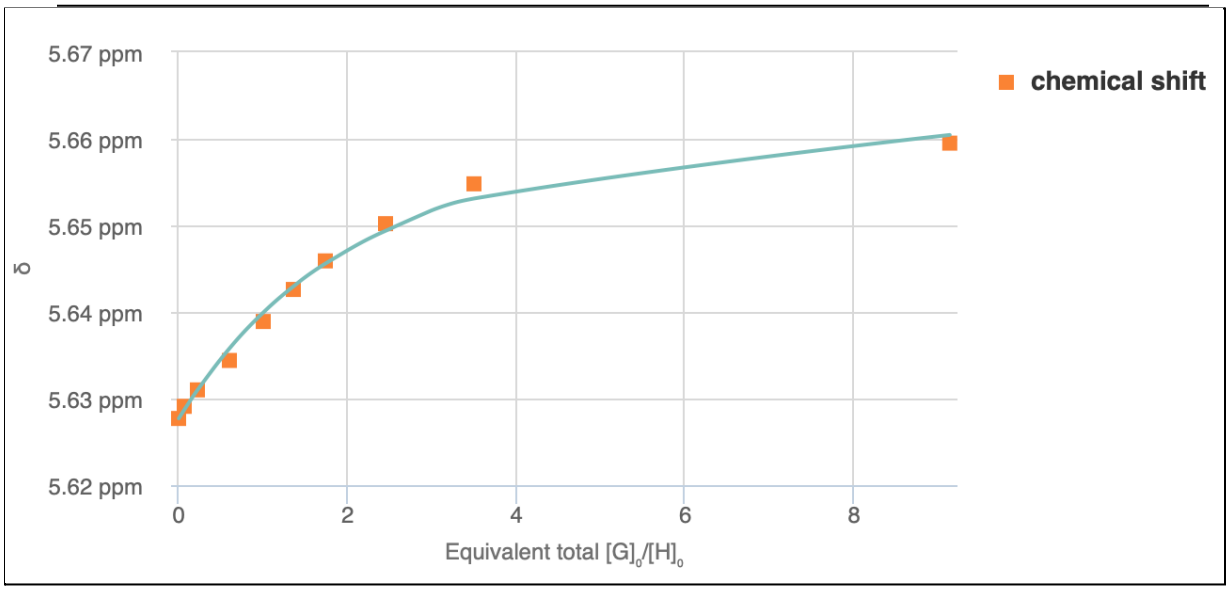

Figure 11. The fit of chemical shift (ppm) against guest concentration (M) over host 2 concentration (M) with K^+ as the guest.

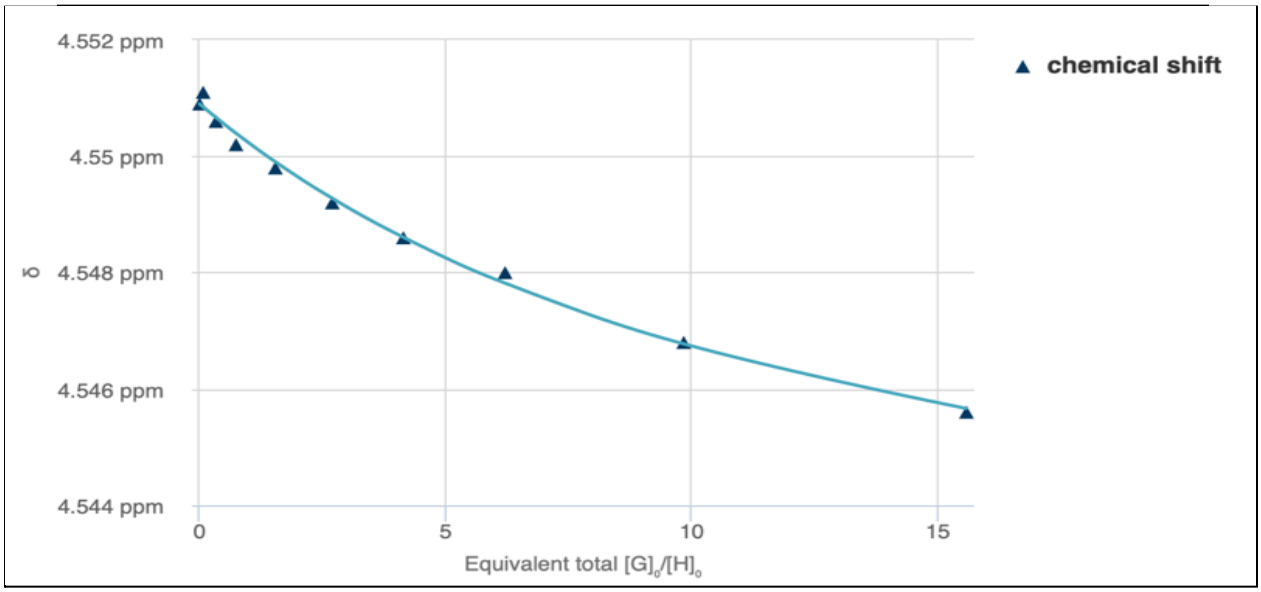

Figure 12. The fit of chemical shift (ppm) against guest concentration (M) over host 1 concentration (M) with Na^+ as the guest.

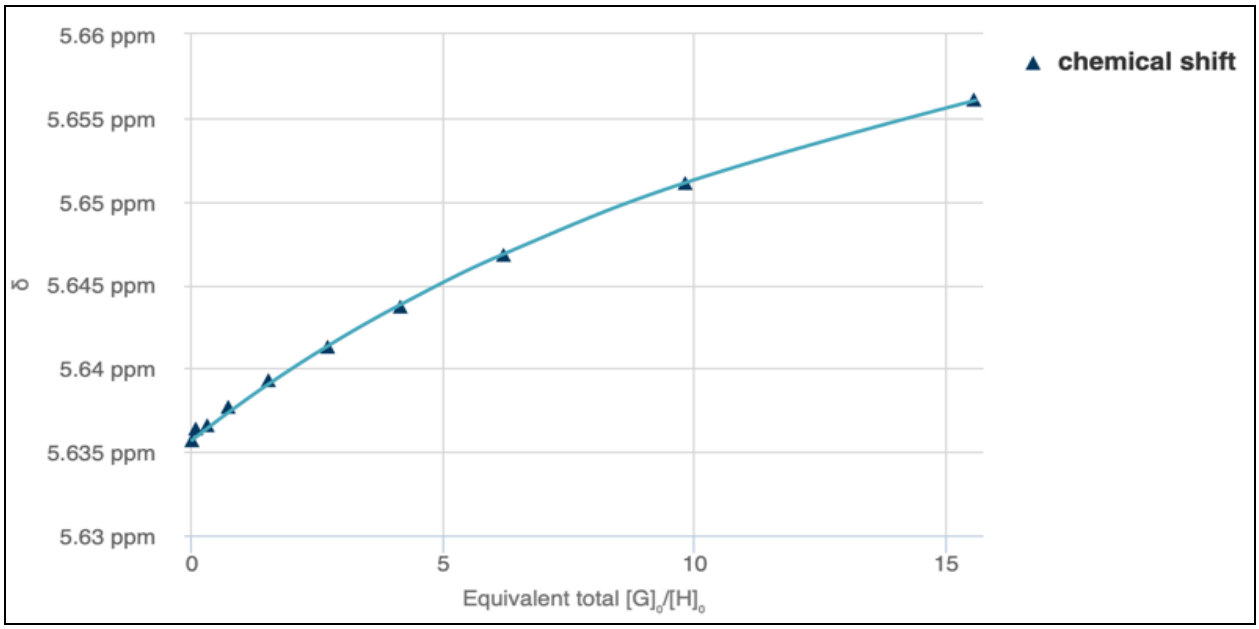

Figure 13. Fit of chemical shift (ppm) against guest concentration (M) over host 2 concentration (M) with Na^+ as the guest.

	Association Constant (M^{-1})	Error
Host 1 & Guest	24.60	± 6.3264%
Host 2 & Guest	49.98	± 13.5864%

Table 3. Association constants & errors in deuterated acetonitrile with K^+ as the guest.

	Association Energy
Host 1 & Guest Complex	$\Delta G = -RT\ln K_a$ $= -(8.314 J*K^{-1}mol^{-1})(298.0K)\ln(24.60)$ $= -7.935 kJ/mol$ $= -1.897 kcal/mol$
Host 2 & Guest Complex	$\Delta G = -RT\ln K_a$ $= -(8.314 J*K^{-1}mol^{-1})(298.0K)\ln(49.98)$ $= -9.691 kJ/mol$ $= -2.316 kcal/mol$
Difference In Association Energy Between The Two Complexes	$\Delta G = (-2.316 kcal) - (-1.897 kcal)$ $= -0.419 kcal/mol$

Table 4. Association energy with K^+ as the guest

	Association Constant (M^{-1})	Error
Host 1 & Guest	11.24	± 7.9156%
Host 2 & Guest	7.95	± 3.0558%

Table 5. Association constants & error in deuterated acetonitrile with Na^+ as the guest.

DISCUSSION

From the proton NMR spectra, it was observed that each addition of the guest changed the chemical environment of the host compounds, and therefore, the chemical shifts of the protons on the host molecules were seen to alter as well. The *Bindfit* program, along with the obtained chemical shifts and the corresponding concentrations of the host and the guest, generated the association constants for the two host and guest complexes. With K^+ as the guest, the host 1 and guest complex had an association constant of 24.60 M^{-1}, and the host 2 and guest complex had an association constant of 49.98 M^{-1}. This signified that host 2 had a higher

interaction with the K^+ guest. The structures of both hosts are very similar, the only difference being the number of aromatic rings. The host molecules were purposely synthesized to have certain identical structural properties such as the number and location of the oxygen and carbon atoms of the crown ethers. Because of this, the effect of the aromatic rings was solely displayed. Host 2 resulted in a higher association constant, which is explained by the structure of the molecule; host 2 had more aromatic rings that were part of the crown ether than host 1. Aromatic rings have π electrons, and since host 2 had a greater number of aromatic rings, it also had a greater amount of π electrons or greater π-electron density; this allowed for a greater cation-aromatic or cation-π interaction, resulting in more potassium ions to interact with the host and form a higher concentration of the host-guest complex. On the other hand, with Na^+ as the guest, the host 1 and guest complex had an association constant of 11.24 M^{-1}, and the host 2 and guest complex had an association constant of 7.95 M^{-1}. These values were very close to each other as the ratio of the two association constant values is close to one, while the ratio of the association constants was slightly over two for the K^+-host complexes. This signifies that the host compounds are less selective towards the sodium ions as both hosts are almost equally interacting with the sodium ions. This is thought to be due to the smaller size of the sodium ions, which causes the Na^+ cations to interact more with oxygens in the compound and have less interaction with the aromatic rings. This makes the host compounds less selective towards Na^+ as both hosts almost have an equal amount of interactions with Na^+. The potassium ions, on the other hand, are larger; they have interactions with the oxygen atoms of the compound, but also are more exposed to interacting with the aromatic rings due to their larger ionic radius. As a result, the K^+ interacts with the aromatic rings more so than Na^+, making the hosts more selective towards K^+. Since the aromatic rings are better able to interact with potassium ions in both hosts,

π-electron density was the factor that differed the interaction of host 1 and host 2 with the potassium ions.

Moreover, the association energy was calculated for both complexes with K^+ as the guest. The host 1 and guest complex had an association energy of -1.897 kcal/mol, and the host 2 and guest complex had an association energy of -2.316 kcal/mol, with a difference being -0.419 kcal/mol. The binding of the guest ion to the host molecule was a spontaneous reaction and was exergonic; the energy of the host decreased because the binding of the guest is a stabilizing interaction as a more stable conformation is achieved with the binding of the guest cation. Host 2 had a higher association energy because of the higher association constant, which informed that host 2 was stabilized by the guest more than host 1 was. Furthermore, the *BindFit* program provided error values for the determination of the association constants. The software has chemical shift and concentration ratios programmed within it in order to compare and validate experimental data. With K^+ as the guest, the association constant for host 1 had an error of ± 6.3264 %, and the association constant for host 2 had an error of ± 13.5864 %. With Na^+ as the guest, the association constant for host 1 had an error of ± 7.9156 %, and the association constant for host 2 had an error of ± 3.0558 %. This error was largely due to the utilized syringes. The syringes were manual, and therefore, obtaining the exact measurements for the titration was extremely difficult and was not always done, as indicated by the error. Also, the syringes sometimes had tiny air bubbles inside them which could not be removed even with great effort; this also had introduced some error.

CONCLUSION

Crown ether compounds have a variety of uses. Increasing solubility and nucleophilicity, mimicking enzymatic functions, and storing ions are just a few of the incredibly useful properties

of crown ethers. Therefore, studying these compounds is quite important. These uses all require researching and learning the interactions of these compounds; the most notable interaction of crown ethers is the association with metal ions. Both host 1 and host 2 favored the binding of the potassium ion, with host 2 having a higher association constant (49.98 M^{-1}) than host 1 (24.60 M^{-1}). Contrarily, the sodium ion bound to both hosts almost equally with little difference, as seen by the association constants of 11.24 M^{-1} for the host 1-Na^+ complex and 7.95 M^{-1} for the host 2-Na^+ complex (which had a ratio close to one for the association constants). This signified that the host compounds are less selective towards the sodium ions and more selective towards the potassium ions. Additionally, there was some error in determining these association constant values: ± 6.3264 % for the host 1-K^+ complex, ± 13.5864 % for the host 2-K^+ complex, ± 7.9156 % for the host 1-Na^+ complex, and ± 3.0558 % for the host 2-Na^+ complex; these deviations were due to the flaw of the manual syringes. Moreover, with K^+ as the guest, host 2 had a greater affinity for the guest because of the additional π-electrons available for a greater cation-aromatic or cation-π electron interaction. This interaction was stabilizing as shown by the spontaneity and by the energy release. Host 2 was stabilized more than host 1, and host 2 had a higher release of energy (-2.316 kcal/mol for host 2 and -1.897 kcal/mol for host 1) because of the higher association constant. Also, with Na^+ as the guest, the two hosts almost had the same affinity for the guest as fewer aromatic-cation interactions were occurring with the host and guest molecules. As mentioned before, deploying resources to study these host-guest complexes is vital because of the multiple ways these compounds can be utilized in both biology and chemistry. Gathering more information through research will increase the efficiency of the uses of crown ethers and make a greater contribution to the fields of science.

SAFETY CONSIDERATIONS

In this experiment, standard organic safety protocol was followed by wearing goggles, gloves, and lab coats. The potassium hexafluorophosphate and sodium hexafluorophosphate compounds were handled with extreme care as they may cause chemical conjunctivitis with contact with eyes, skin irritation with contact with skin, gastrointestinal irritation with nausea, vomiting, and diarrhea if ingested, and respiratory tract irritation if inhaled [7,8]. When working with an NMR spectrometer, it is important to keep in mind the recommended restrictions provided by the manufacturer. Metallic objects must not be brought within 10 feet of the magnets and no objects can be placed inside the NMR except for the NMR tube and the holder. People with medical implants such as cardiac pacemakers and neurostimulators also need to stay away from the NMR; exposure could result in injury or death [1]. Another hazard of working with NMR spectroscopy is the fragility of the NMR tubes. These tubes have thin walls and can easily break; therefore, it is important to be careful when placing them into the NMR to prevent injuries.

ACKNOWLEDGMENT

I would like to thank Dr. Bright Emenike for providing the necessary materials and instrumentation required for the execution of these experiments. This project would not have been possible without his guidance and support.

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Investigating Estrogen Effects on Microglia and Glioblastoma Function

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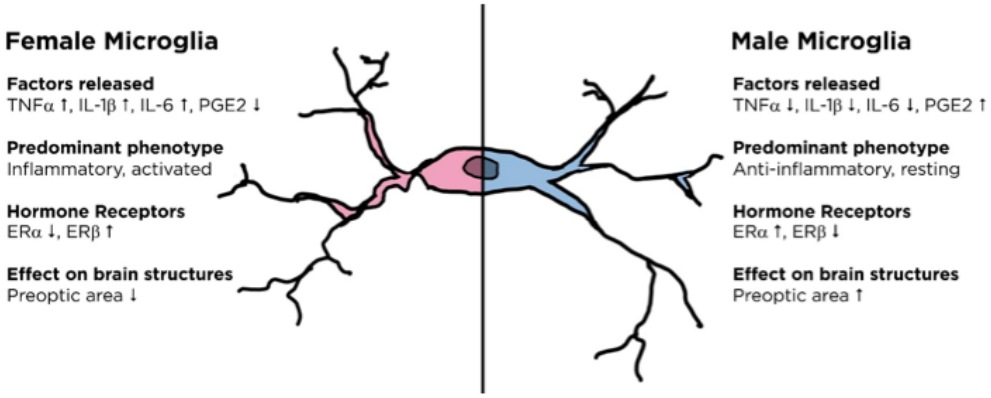
ABSTRACT

Glioblastoma multiforme (GBM) is the most common and most aggressive primary tumor of the brain, and is associated with one of the worst 5-year survival rates among all human cancers. Extensive analysis of patient data has shown a biological sex-based disparity in GBM, reporting that males are 1.5 to 3.5 times more likely to develop brain tumors than females, and subsequently have more extensive tumor necrosis and reduced survival compared to females. Contributing to the progression of this disease are the resident immune cells of the brain and spinal cord known as microglia. Microglial cells are inexorably linked to GBM, as they can comprise over 30% of cells found in glioma biopsies. While in inflammatory microglial function in an anti-tumorigenic manner, gliomas can disrupt this by releasing factors that polarize microglia to an immunosuppressive phenotype, which in turn secrete cytokines that support tumor growth and spread. Therefore, a shift in microglial populations towards more pro- or anti-inflammatory behavior could greatly impact GBM progression. As the sex-linked steroid hormone estrogen is more prevalent in females, we hypothesized that estrogen may play a role in promoting a pro-inflammatory shift in microglial populations, as well as function in a suppressive manner towards glioblastoma cells. Interestingly, we found that estrogen polarizes microglia to anti-inflammatory phenotype, but suppresses GBM migration. These conflicting results have refocused our future work on other genetic mediators of microglial function.

INTRODUCTION

The central nervous system (CNS) is separated from the rest of the body by the blood-brain barrier, which prevents the peripheral immune system from gaining access to the brain and spinal cord [7]. Therefore, the central nervous system has its own local innate immune cells called microglia (10-15% of the cells within CNS) [7]. Microglia function in a similar manner to peripheral macrophages; they protect the central nervous system mainly through the process of phagocytosis [7]. When the CNS is damaged or infected, the microglia become activated and will increase in number and undergo a variety of genetic, morphological, or functional changes [8].

Activated microglia fall upon a spectrum of function states, such as M1 and M2 microglia [12]. M1 microglia can be induced by LPS (lipopolysaccharide) to release factors such as TNFα (a pro-inflammatory cytokine) and nitric oxide, signaling molecules that promote inflammation [2,8]. This inflammatory response is associated with autoimmune diseases that are predominantly seen in females [2,6]. M2 microglia, on the other hand, can be induced by the anti-inflammatory cytokine IL-4 to trigger the release of more anti-inflammatory cytokines (IL-10 and TGF-β), which further induces cell proliferation and migration of brain tumor cells [2,3,8,9,10].



Nissen, J. (2017). *Gender-related disparities in microglial function* [Image]. International Journal of Molecular Sciences.

Glioblastoma Multiforme (GBM) is the deadliest tumor that affects the nervous system. Studies and clinical diagnoses have shown males are more vulnerable to GBM than females [8]. The average survival rate for a person diagnosed with this cancer ranges from 10-12 months, and radio and chemotherapy have proven to be insufficient in the treatment of this disease due to side effects that arise from these treatments [7]. Therefore, pharmacological treatments are needed in order to combat this cancer by targeting the M2 microglia that are highly associated with the proliferation of the cancer cells [7]. Previous research has shown that the pro-inflammatory characteristics of M1 microglia aid in reducing the effects of GBM [7]. Since females have more M1 microglia (pro-inflammatory) than males, a sex-based disparity can be seen in microglia with regards to their susceptibility to GBM. This discovery motivated researchers to understand the importance of estrogen, a sex-linked steroid hormone found predominantly in females, in neuroprotective functions when dealing with brain tumors such as GBM [4]. Although these findings are significant, the exact mechanism by which estrogen mediates neuroprotective functions is yet to be determined [4].

Therefore, our research focuses on the use of estrogen when observing the wide-spread growth of GBM [8]. We hypothesized that estrogen could induce microglia towards a pro-inflammatory state, which could subsequently inhibit glioblastoma cell proliferation and migration. Focusing on sex-dependent differences in microglial populations that lead to tumor proliferation and growth can ultimately lead to new strategies that may be more effective in treating patients afflicted with GBM.

RESULTS

Estrogen treatment suppresses inflammatory cytokine release by microglia

The N9 microglial cells were grouped into two categories: Control (without estrogen) and β-estradiol (with estrogen). These groups were either untreated or treated with LPS (inflammatory polarized) or IL-4 (anti-inflammatory polarized). The RNA of these conditions were collected to analyze for markers of inflammation. RT-PCR was performed to transform the mRNA into cDNA to study the effects of either LPS or IL-4 on the treated N9 cells. Gel electrophoresis was subsequently performed to visualize these effects via the measurement of TNFα expression (**Figure 1A**). The addition of LPS pushed the N9 cells towards an inflammatory state, concluded by the increase in TNFα fold-change (**Figure 1B**). However, surprisingly a decrease in inflammatory response was observed when β-estradiol treated LPS was added to the cultures with a significant decrease in TNFα expression (**Figure 1B**). Similar effects were seen in Control Il-4 and β-estradiol treated IL-4.

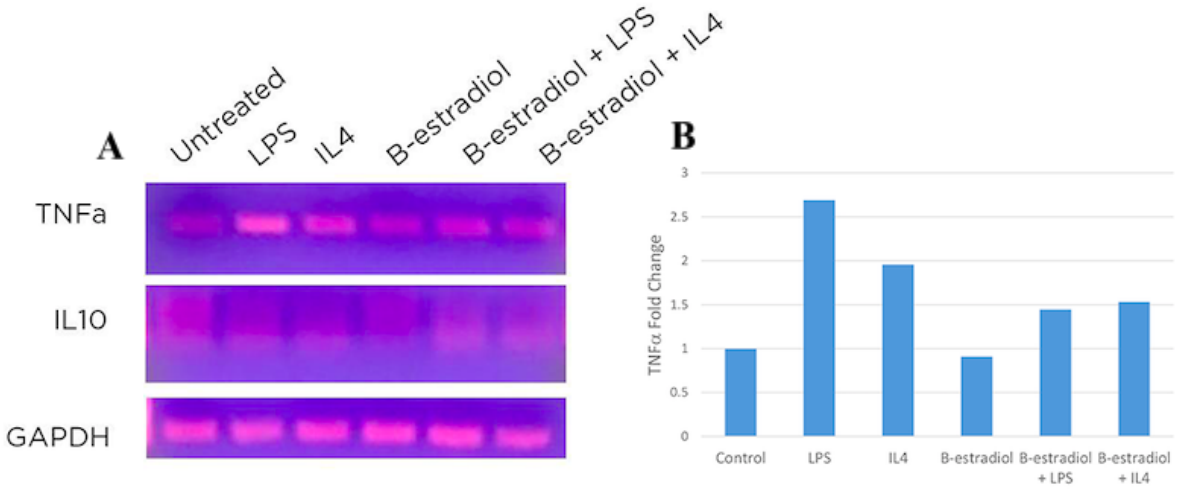
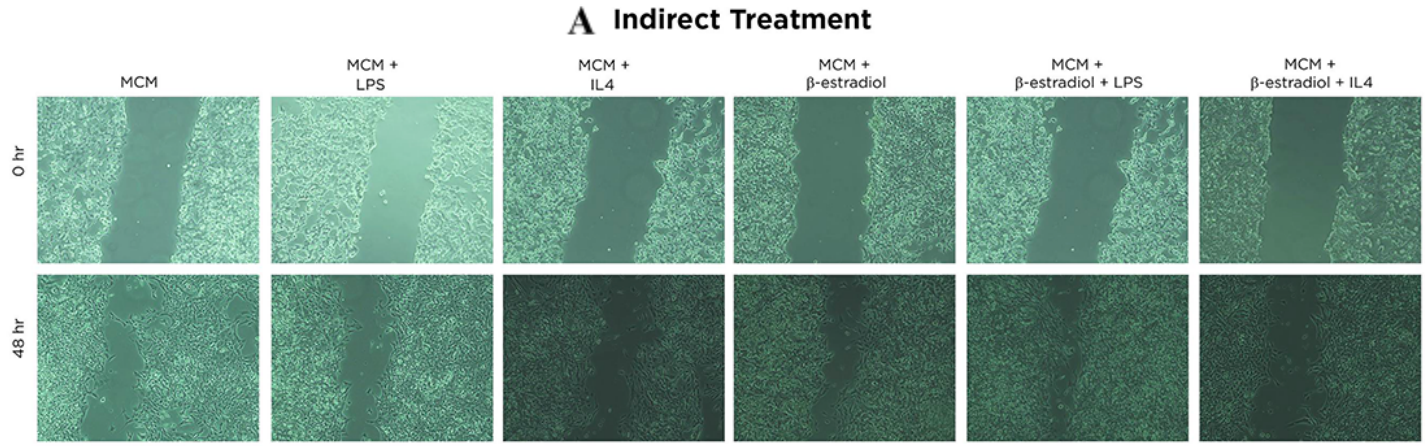


Figure 1: The N9 microglial cells were placed in a 3x2 well plate and treated with the following conditions: untreated, LPS, IL-4, β-estradiol, β-estradiol + LPS, and β-estradiol + IL-4. RNA was harvested from these cells after a 24-hour treatment and then reverse transcribed into cDNA. [**A**] The cDNA was analyzed for TNFα (pro-inflammatory), IL-10 (anti-inflammatory), and GAPDH

(control) gene expression. [**B**] The bar graph on the right depicts the quantification of the fold change of TNFα levels.

Indirect and Direct estrogen exposure effects on the migration and proliferation of GL261 cells

To observe the indirect effects of the factors N9 cells release on the GL261 glioma cells' migration and proliferation, the microglial conditioned medium (MCM) was filtered to remove the treated N9 cells, harvested, and transferred to the cancer cells. The scratch assay was performed to observe migration and the MTT assay was performed to observe proliferation (**Figure 2A**). MCM treated control conditions showed an increase in migration after 48 hours. However, the MCM-treated β-estradiol conditions also showed increased migration in the GL261 cells. Furthermore, the indirect treatment results were unclear in pointing out whether or not proliferation occurred (**Figure 2B**).



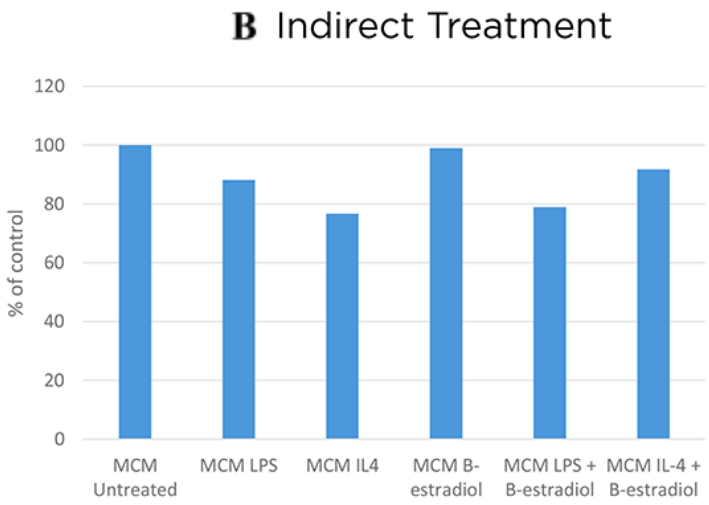
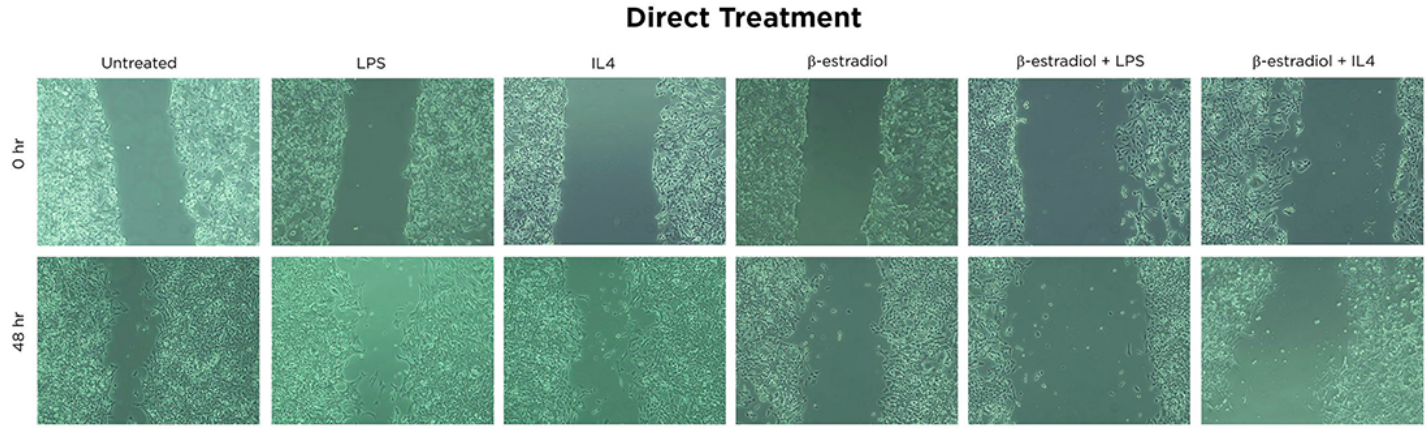


Figure 2: The conditioned media isolated from treated N9 cells (MCM) was harvested to treat the GL261 glioblastoma cells. A scratch assay was performed and followed by a washing procedure to detach loose cells and the culture media was swapped to the appropriate treatment. [**A**] The cancer cells were then analyzed over 48 hours to observe for migration. [**B**] Cell survival and proliferation were further determined by MTT assay.

To observe how estrogen directly affected cancer cells, the GL261 cells were treated with the same exact treatment used on the N9 cells but instead were directly treated with estrogen. The scratch assay and MTT assay methods used in the indirect treatment were also applied for this treatment. The β-estradiol conditions showed a reduction in the migration of the GL261 cells (**Figure 3A**). Furthermore, the proliferation of the GL261 cells was also reduced as seen in **Figure 3B**.



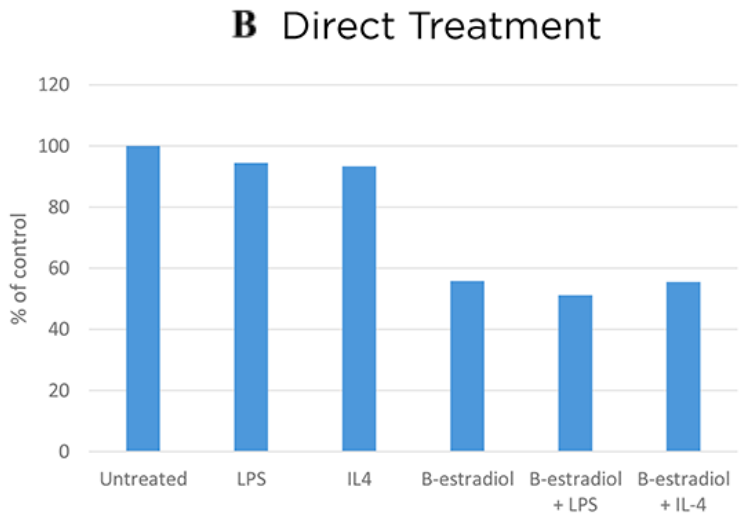


Figure 3: The GL261 glioblastoma cells were directly treated with estrogen. A scratch assay was performed and a subsequent washing was performed to detach loose cells and the culture media was swapped to the appropriate treatment. [**A**] The migration of the cancer cells was analyzed after 48 hours. [**B**] Cell survival and proliferation were further determined by MTT assay.

DISCUSSION

Pro-inflammatory characteristics of M1 microglia have shown to have reducing effects on GBM, as females, found with more pro-inflammatory microglia (M1), have a lower incidence of developing GBM than men, who have more anti-inflammatory microglia (M2) [5,7,8]. Based on these facts, we hypothesized that estrogen would be able to polarize the microglial cells to an inflammatory state, which would further help inhibit the migration and proliferation of GL261 cells. Previous studies have suggested that estrogen has a significant tumor-suppressive role on GBM via estrogen nuclear receptors (ERα and ERβ) [5,13]. Unlike ERα, ERβ is considered a tumor suppressor as its expression decreases GBM progression by reducing cell proliferation and inducing apoptosis in the cancer cells [5,11]. Therefore, our study implements the use of β-estradiol as the form of estrogen treatment for the N9 and GL261 cells.

Our results contradicted our hypotheses as they (1) displayed that estrogen treatment had an inhibitory effect on the microglial cells by reducing their inflammatory expression of TNFα after LPS stimulation (**Figure 1**) and (2) estrogen-polarized microglia rather exhibited an increased migration and unclear proliferation in the GL261 cells (**Figure 2**). Based on these results, it can be assumed that the addition of β-estradiol rather pushed the microglial cells towards an anti-inflammatory state, which resulted in the consequent increase in migration of the GL261 cells. The GL261 cells were also directly treated with β-estradiol. The results for the direct treatment were more in accordance with the hypothesis that estrogen suppresses the migration and proliferation of GL261 cells (**Figure 3**). This further concluded that the direct treatment was more effective and successful in suppressing the GL261 cells migration and proliferation compared to the indirect treatment.

CONCLUSION

This study concludes that estrogen exposure to the glioma cells (direct treatment) suppressed the migration and proliferation of the cancer cells, whereas the estrogen-polarized microglia exposure to glioma cells (indirect treatment) had the opposite effect. We had hypothesized that estrogen would be able to polarize the microglial cells to an inflammatory state. However, we found that estrogen suppressed the release of inflammatory factors rather than increasing the release of inflammatory factors, concluding that estrogen does not polarize microglial cells to an inflammatory phenotype. Furthermore, the indirect treatment resulted in the stimulation of glioblastoma migration, which also contradicted our hypothesis that estrogen-polarized microglia would further suppress the migration and proliferation of glioma cells. These results have focalized future research to understand that perhaps it is not estrogen's immediate effect on microglial cells that mediates this biological sex-based difference. Rather,

exposure to estrogen during development could possibly initiate genetic changes in the microglial populations that could further change the way microglial cells react to disease/cancer.

FUTURE DIRECTIONS

Based on the study regarding RNA Sequence Analysis, the next stage of our research focuses on the genetic disparities through analysis of differential expression of biological sex-specific genes [12]. Previous research has shown that Nrp1, a transmembrane co-receptor found in the microenvironment of gliomas, plays a significant role in altering the immune cells of the central nervous system, making them immunosuppressive [1]. Therefore, a positive correlation was found between Nrp1 and glioma grade, as high-grade gliomas had high Nrp1 expression [1]. Our future research looks to observe the direct effects of Nrp1 not only on microglial polarization, but also on glioblastoma cell proliferation and migration. Moreover, we also plan to observe the effects of the male primary sex hormone, testosterone, on microglial polarization as well as GB cell proliferation and migration.

ACKNOWLEDGEMENTS

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Finding Cosmic Strings with Black Hole-Pulsar Binaries

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ABSTRACT

Cosmic strings are theorized to have formed due to symmetry breaking during a phase transition of the early universe. A cosmic string can be captured by a black hole, which can result in the black hole losing its rotational energy as it is transferred to the string. This loss of energy can be detected by observing a pulsar rotating around the black hole. As the mass of the black hole decreases, the pulsar outspirals, and its overall orbit increases. Based on an energy extraction rate formula, we can determine how long it takes for a black hole to lose all its energy and how large this effect is on the period of a pulsar. Through this method, we demonstrate that black hole energy loss via cosmic strings is indirectly observable by pulsar observation.

INTRODUCTION

The topological defects or cracks that are observed in ice as water undergoes a phase change are analogous to the topological defects that may have occurred on a cosmological scale in the early universe. Cosmic strings are theorized to be the products of these topological defects as the universe cooled down. Since cosmic strings are relativistic strings, they are completely characterized by the tension μ, which is defined as the mass per unit length of the cosmic string [11]. The units of this tension are the same as the Planck units $G_N\mu/c^2$ but with a slight change due to the fact that both G_N and c^2 are equal to 1; therefore, the units for the tension is simply μ [7]. A μ value of 10^{-15} or greater has already been ruled out by Laser Interferometer Gravitational-Wave Observatory (LIGO) [1]. The cosmic strings may be extremely thin, but are also extremely dense; thus, when they vibrate, their gravitational waves can be detected [12]. As the universe continued to expand, it is likely that these strings expanded or perhaps collided with one another or even other celestial objects such as black holes [12]. Gravitational-wave detectors have been successful in detecting the gravitational energy formed by black holes and neutron star collisions but have been unsuccessful in detecting potential waves from cosmic strings. Variations in pulsar signals, however, may indirectly indicate the existence of cosmic strings [12]. This research looks to theorize the likelihood of a cosmic string being found attached to (or collided with) a black hole. An illustration of a rigidly rotating cosmic string attached to a black hole is shown below [7].

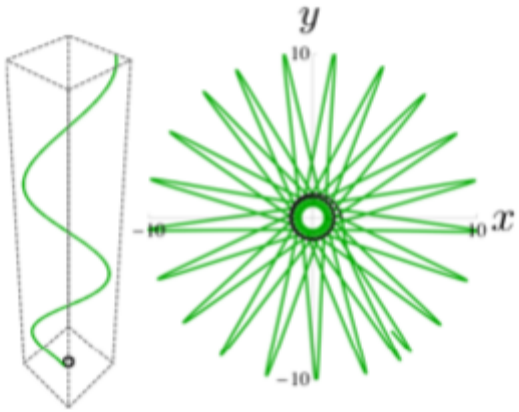

Figure 1. The left image shows the black hole at the bottom while the green line represents the string [7]. The right image illustrates how this would look if one were observing a rotating cosmic string from above [6].

Cosmic strings can extract energy from a black hole by means of the Penrose Process. This theory states that the energy of a rotating black hole can be extracted from it as long as the object extracting the rotational energy is located in the ergosphere of the black hole [8]. Any object that falls within the ergosphere will begin rotating with the black hole and could potentially split into two parts [8]. One part of the object will go through the event horizon and completely fall into the black hole; however, the other part could escape the black hole and leave with greater energy than what it originally had [8]. A decrease in the angular momentum of the black hole leads to the transfer of some angular momentum to the escaping part of the object [8]. Based on the mass-energy equivalence formula, the black hole loses energy and mass as an object escapes. This type of energy extraction can be defined as the Penrose process and according to this process, the black hole can only lose a maximum of 29% of its mass [8]. A cosmic string, like any particle, can fall within the ergosphere of the black hole and can extract the rotational energy of the black hole. The Penrose process makes a black hole-cosmic string collision unique because if a cosmic string were to interact with any other celestial object, it would most likely move past it

after the collision. However, in the case of a black hole, the cosmic string can potentially be captured by the event horizon of the black hole and become stuck to it [8].

Changes in the mass of a black hole containing a cosmic string can be detected by observing a pulsar orbiting the black hole. Pulsars are considered to be one of the most precise clocks of space, as they are rotating magnetized neutron stars that release electromagnetic waves from their magnetic poles. The waves emitted from these pulsars can only be detected when the pulsar's magnetic pole is facing toward the earth through the use of ground-based radio telescopes and X-ray/Gamma-ray satellites [3,9].

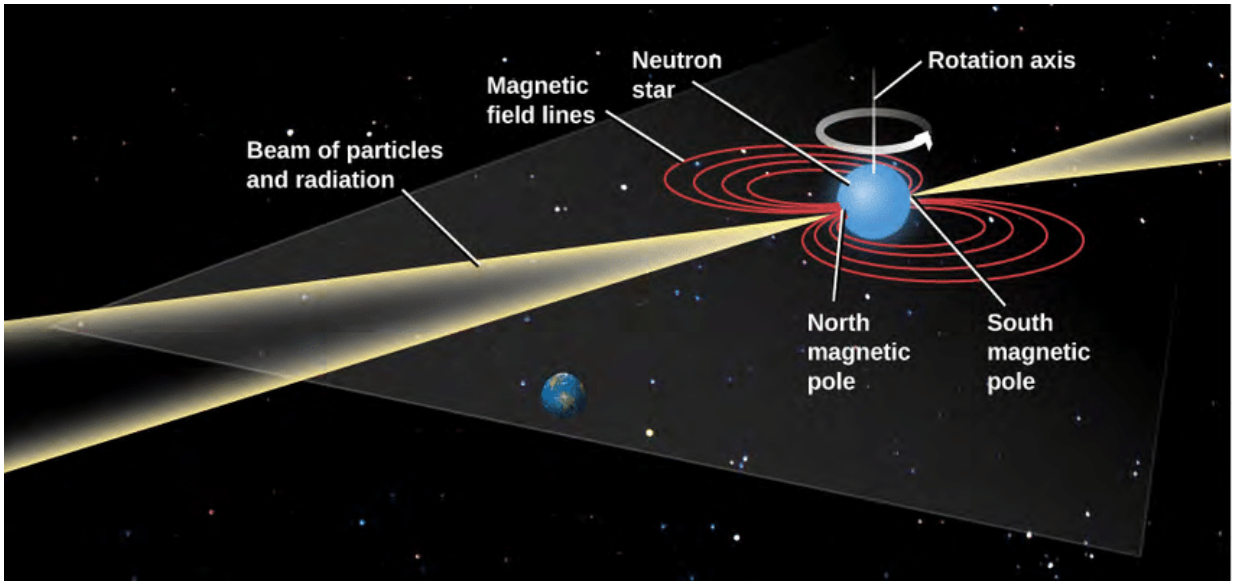

Figure 2. Fraknoi et al. (2020). *Model of a pulsar* [Image]. Astronomy.

Since pulsar-neutron star binary systems have already been observed, it would not be far-fetched to observe a pulsar rotating around a black hole [6]. With the advent of new instruments such as the Square Kilometer Array and improved torsion-balance measurements, it is likely that black hole-pulsar binaries will be detected in the near future [3,9]. Our research, therefore, looks to

use these black hole-pulsar binaries to observe black hole energy loss due to black hole-cosmic string collisions.

SETTING PARAMETERS FOR A BLACK HOLE-COSMIC STRING COLLISION USING PULSAR-BLACK HOLE BINARIES

According to Kepler's Third Law, the orbital period *P* (time taken by a pulsar to orbit a black hole once) of the pulsar is related to the orbital semi-major axis *a* from the black hole and the black hole mass *m*. The formula is as follows:

$$P^2 \propto a^3 \qquad \textbf{(1)}$$

This equation states that P, the orbital period of the pulsar squared, is proportional to the semi-major axis of the black hole cubed. A derivation of Kepler's Third Law where the mass of the black hole is assumed to be slowly varying is as follows:

$$\dot{P}_{ML} = -2P\frac{\dot{m}}{m} \qquad \textbf{(2)}$$

This equation states that for a given mass-loss rate of a black hole, there is a change in period [10]. Any changes in the orbital period of the pulsar would indicate changes in the mass-energy loss of the black hole. Since the orbital period of the pulsar can be measured, the mass loss that occurs within a black hole containing a cosmic string can also be detected. If both sides of this equation were divided by P, the equation would be as follows:

$$\frac{\dot{P}_{ML}}{P} = -2\frac{\dot{m}}{m} \qquad \textbf{(3)}$$

$\dot{P}$ is the minimum rate of change of the orbital period of the pulsar and $\dot{m}$ is the minimum black hole mass-loss rate that can be observed, whereas, P is the period of the pulsar and m is the mass of the black hole [10]. Thus, the fractional change in the period is the same as the fractional change in the mass of the black hole [10]. The negative sign indicates that as the mass decreases, the period increases. The formula can then be rearranged to solve for $\dot{m}$ as follows:

$$\frac{-(m)(\frac{\dot{P}_{ML}}{P})}{2} = \dot{m} \qquad \textbf{(4)}$$

The rate of change that has been observed for the orbital period of the binary pulsar is $\dot{P} = 10^{-12}$ s/s while the period of the pulsar is P = 10 hrs [10]. We assume the pulsar is orbiting a stellar-mass black hole with $m = 3M_{\odot}$, where $M_{\odot}$ is solar mass. These values were inserted into the formula to give a minimum detectable mass-loss rate of $10^{-16}M_{\odot}$/s.

This minimum mass-loss rate of the black hole can be compared with the energy extraction rate of a black hole that has captured a cosmic string [7]:

$$\frac{dE}{dt} = 5.8 \times 10^{51}\text{erg/s}\left(\frac{G_N\mu/c^2}{1.3 \times 10^{-7}}\right) \qquad \textbf{(5)}$$

The relevant value here is that of μ, the tension of the black hole. It can be seen from this formula that the tension of the cosmic string is proportional to the energy-extraction rate. Thus, the higher the tension of the cosmic string, the more energy that is extracted from the black hole.

This formula was manipulated so that the minimum mass-loss rate of the black hole could be substituted for the energy extraction. 5.8×10^{51} erg/second was converted to $M_{\odot}$/second resulting in a value of 3×10^{-3} $M_{\odot}$/second. The formula was isolated as such:

$$\frac{d\dot{m}}{dt} = 3 \times 10^{-3} M_{\odot}/s \left(\frac{\mu}{1.3 \times 10^{-7}} \right) \quad \textbf{(6)}$$

The minimum mass loss rate of 10^{-16} $M_{\odot}$/s was inserted into the equation to give a minimum detectable tension of $\mu = 10^{-20}$. This result of μ is of particular interest since the tension of a cosmic string being 10^{-15} or greater has already been ruled out by the observations made by LIGO. Based on these results, the following graph illustrates the ranges at which a black hole-cosmic string collision can possibly be observed.

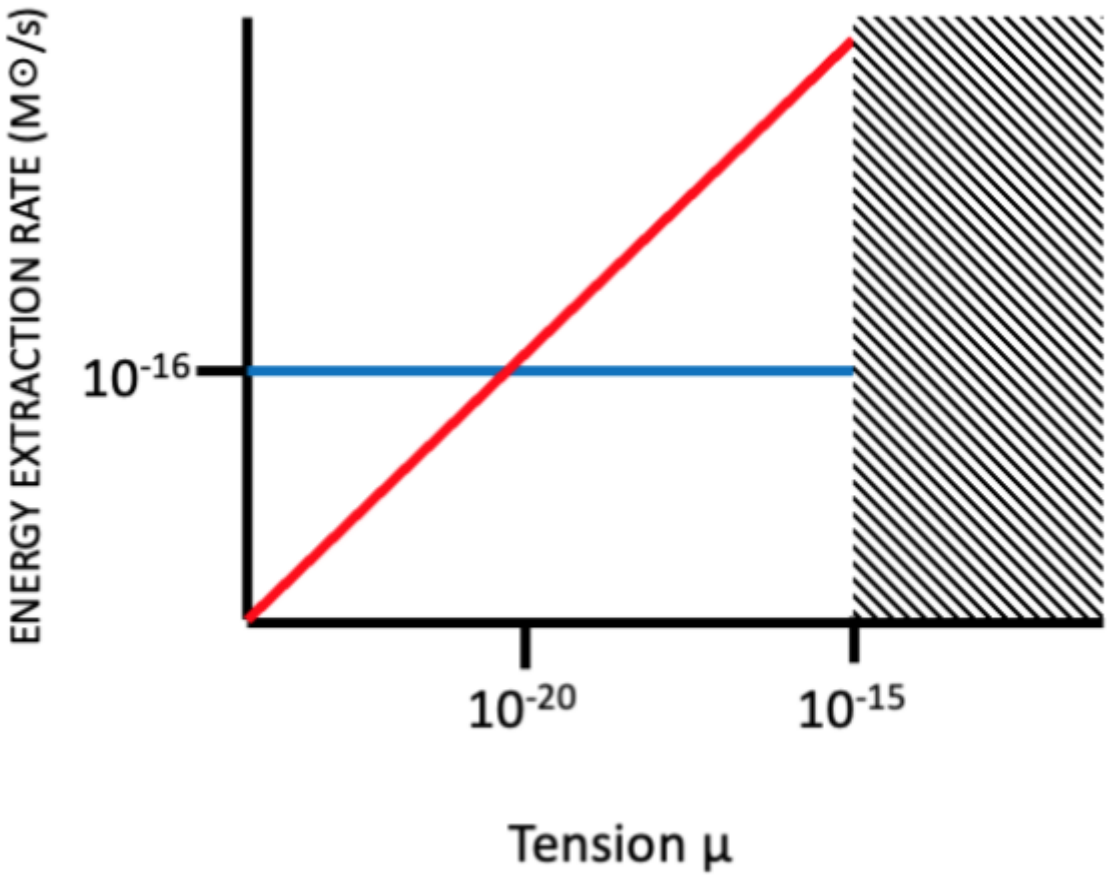

Figure 3. Energy extraction rate and cosmic string tension ranges allows for the observation of black hole-cosmic string collisons

The vertical axis in **Figure 3** represents the energy extraction rate while the horizontal axis represents the tension of the cosmic string μ. The minimum energy extraction rate that can be observed is 10^{-16} ($M_{\odot}$/s). The tension value of 10^{-20} represents the minimum tension of the cosmic string needed in order to observe black hole energy transferred to a cosmic string. On the other hand, the tension values equal to or greater than 10^{-15} represent the tension values that have been ruled out by the LIGO [1].

CONCLUSION

A black hole-cosmic string collision is a theorized phenomenon, but its likelihood is not far-fetched [2,5]. In fact, cosmic strings could have been detected in the Milky Way's galactic center, which is a supermassive black hole [2,5]. A filament has already been discovered at the central black hole of the galaxy and is being analyzed [2,5]. Furthermore, black hole-pulsar binaries may also be detected in these galactic centers [3,9,10]. With the advent of new instruments such as the Square Kilometer Array and improved torsion-balance measurements, the detection of black hole-pulsar binaries is now more promising than ever before [3,9,10].

Based on Kepler's Third Law for a black-hole pulsar binary as well as the energy extraction rate formula for black hole-cosmic string collision, we were able to theorize that a black hole-cosmic string collision can be observed under the circumstances that the energy extraction rate is at a value of or above $10^{-16}M_{\odot}$/s and the tension of the cosmic string is at a value ranging from $10^{-20} < \mu < 10^{-15}$.

Future Directions

Although we were able to determine the ranges at which a black hole-cosmic string can be observed, we have yet to estimate the actual likelihood of such a collision occurring. Our calculations would also rely on an actual pulsar-black hole binary being observed in the future.

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Enhancing the Liposome Uptake of Hydrophobic Porphyrin Using Cyclodextrin

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ABSTRACT

Porphyrins are photosensitive compounds that produce reactive oxygen species (ROS) that are used in photodynamic therapy (PDT) in cancer treatment. Derivatives of porphyrins (e.g. photofrin) are clinically approved as an option for various cancer treatments. As a result, the method for the delivery of porphyrins, such as using liposomes for packaging porphyrin, is an area of ongoing research interest. However, derivatives of hydrophobic porphyrins (e.g. tetraphenyl porphyrin, TPP) tend to self-aggregate in aqueous solutions due to the conjugated pi system that enables the intermolecular pi-stacking interactions. In order to overcome this problem, tetramethyl-B-cyclodextrin (TMe-B-CDx) is used to encapsulate TPP to prevent aggregation and improve the solubility of porphyrins. The loading efficiency of porphyrins in an aqueous solution is improved by the formation of the water-soluble TPP-TMe-B-CDx complex. Different mole ratios have been devised to test the stabilities of liposomes after the loading process, which determined whether complexing porphyrin to cyclodextrin is a viable approach for experimentation.

INTRODUCTION

The use of liposomes to function as drug carriers has been of great interest due to the amphiphilic nature of lipids (or phospholipids). When placed in polar solvents, the hydrophilic 'heads' tend to face the solvent and the hydrophobic 'tails' (composed of fatty acid) interact with each other, forming a uniform bilayer vessel. This self-interacting mechanism results in the formation of a bilayer with a hydrophilic core and a hydrophobic region in between the layers; this is where TPP will then be loaded (**Figure 1**). The functional groups attached to the porphyrin molecules dictate its characteristics and may influence the loading efficiency. (e.g. tetraphenyl-porphyrin (TPP)) (**Figure 2**). The four phenyl rings attached to the porphyrin control the hydrophobic property and the aggregation tendency of the porphyrin in a polar solution. The origin of porphyrin's aggregation can be traced back to the formation of the pi system of the compound, which facilitates pi-stacking interactions [3].

A potential way to interfere with porphyrin from aggregating is packaging it in trimethyl-beta-cyclodextrin (TMe-B-CDx) before loading the liposome. TMe-B-CDx is made up of subunits of glucose in a cyclic or ring conformation with a hydrophobic interior and hydrophilic exterior (**Figure 3**); it is often utilized by pharmaceutical companies for drug packaging or delivery [1,7]. With this, TPP and TMe-B-CDx can bind to each other by hydrophobic interactions within the cavity of the cyclodextrin compound, forming a complex and improving loading of the liposome (**Figure 4**). At a certain wavelength, TPP produces ROS that are capable of degrading the liposome through oxidation to further release its contents. TPP can then accumulate and locate cancer cells; the oxidation reaction occurs once more in order to eliminate the cancer cells (**Figure 5**). Similar to liposomes, the membranes of cancer cells are composed of

phospholipids; therefore the degradation of the cell membrane results in cell vulnerability and eventually death [6].

If successful in loading and stabilizing the liposome, the following procedure would be able to introduce the liposome to cancer cells. Porphyrins are used as a sensitizer clinically for photodynamic therapy (PDT), a form of cancer treatment with fewer side-effects compared to other options, such as radiotherapy and other forms of chemotherapy [6].

Other researchers have tried different techniques for the insertion of porphyrins into the liposome using various methods based on the hydrophilicity or hydrophobicity of the functional groups [3]. The methodologies vary from introducing porphyrin and lipid in the same organic solvent to having an organic solution of porphyrin introduced to an aqueous solution of lipid [3]. Though liposomes can attempt to prevent the occurrence of aggregation, they will be used predominantly for packaging and acting as a vessel. By forming an intermediate complex, it can enable the porphyrin complex and lipid to co-exist in the aqueous environment and enable TPP to integrate within the liposome. Also, porphyrins are not the limit for this reaction to occur. Other modified cyclodextrin compounds can be used to enhance the solubility of other hydrophobic aromatic molecules in order to integrate within liposome drug vessels as well [2,7].

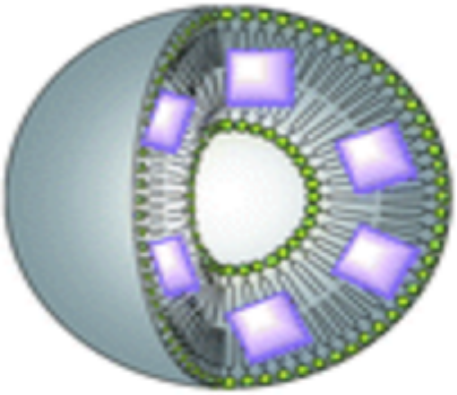

Figure 1. TPP loaded within the hydrophobic bilayer of the liposome

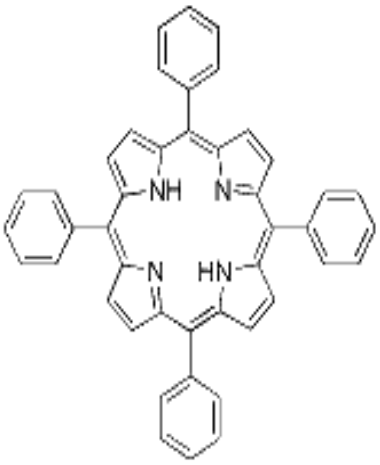

Figure 2. Tetraphenyl-porphyrin (TPP)

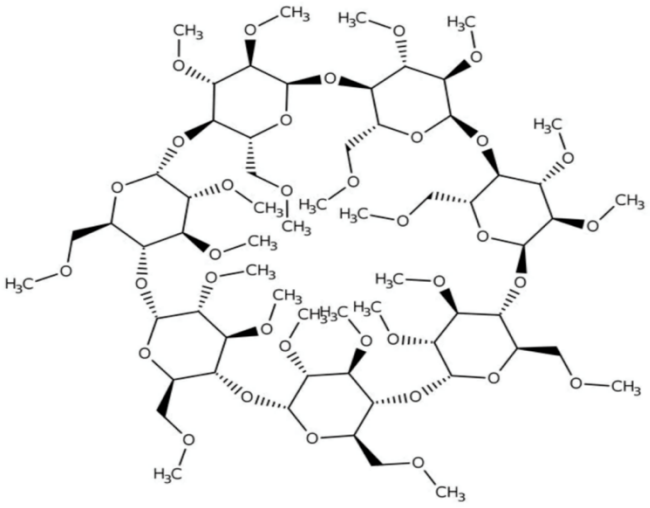

Figure 3. Trimethyl-Beta-Cyclodextrin (TMe-B-CDx)

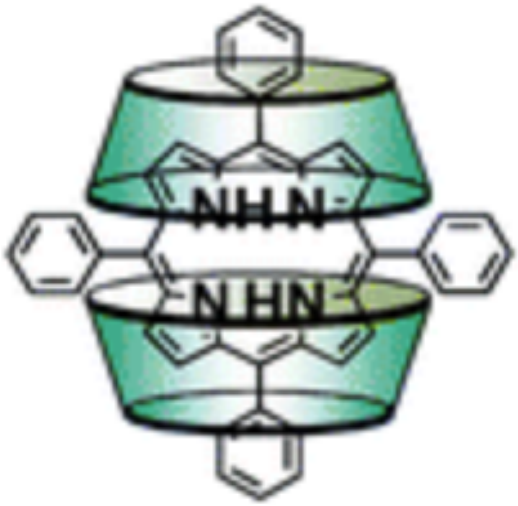

Figure 4. TPP-TMe-B-CDx Complex via hydrophobic interactions within the cavity of the cyclodextrin compound improves porphyrin loading.

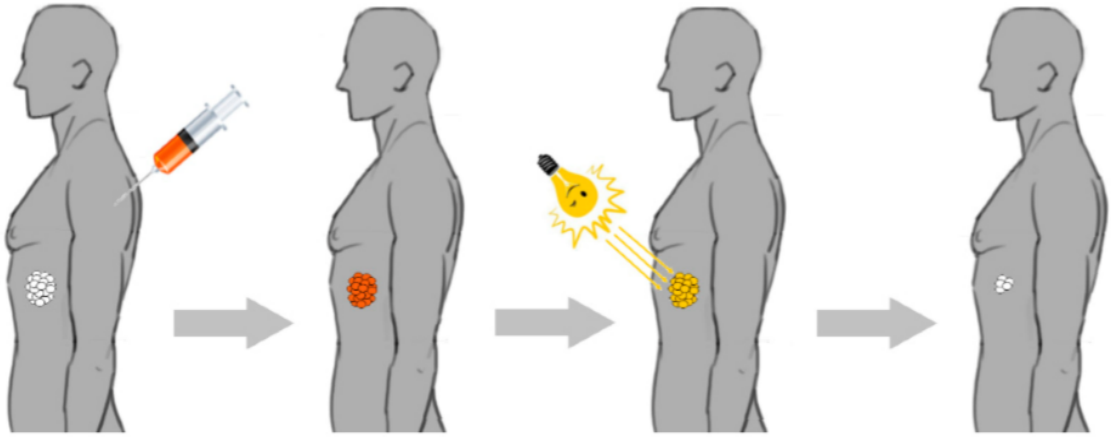

Figure 5. PDT Mechanism
(i) photosensitizer injection; (ii) photosensitizer accumulation in the tumor;
(iii) photosensitizer activation by light; (iv) ROS production and tumor damage response

EXPERIMENTAL

Preparation of the TPP-CDx Complex

0.005 g (8.13×10^{-6} moles) of tetraphenyl porphyrin and 0.0233 g (1.63×10^{-5} moles) of trimethyl-beta-cyclodextrin were added in a microcentrifuge with two mixing balls. Both solids

were mixed vigorously for 20 minutes using a vortex mixer until the resulting mixture was almost a fine powder. The mixing balls were removed and 1.5 mL of pure water was added to the solid mixture to form a dark green solution. The sample was then incubated overnight at room temperature; Alternatively, the solution was centrifuged for 15 minutes at 15,000 rpm. This allows for undissolved TPP to form a precipitate and separate from the solution. A change in color from dark green to purple was observed in the aqueous solution, confirming that the TPP-TMe-B-CDx complex had been formed. The aqueous solution was transferred to a 15 mL test tube and diluted with pure water to a final volume of 10.0 mL. The concentration of TPP in the TPP-TMe-B-CDx complex was determined by measuring the absorbance of the solution at 415 nm in a 1.25 cm cell via UV-Vis spectroscopy. The molar absorption coefficient for the solution was

$\varepsilon_{415\ nm} = 3.308 \times 10^5\ L\ mole^{-1}\ cm^{-1}$.

Preparation of the liposome

In a 20 mL glass vial, a 4 mM stock solution of liposome was made in 15 mL of water by first dissolving 46.5 mg (6.00×10^{-5} moles) of L-a-phosphatidylcholine (Soy PC) with 1mL of chloroform. It was then evaporated to dryness using a vacuum desiccator overnight to form a lipid film that appeared at the bottom of the vial. After desiccation, 15 mL of water was added to the lipid film and mixed thoroughly for 5 min using a vortex mixer. The liposome solution was then stored in a fridge (>0^{o}C) for later use.

Preparation of loading liposome with TPP

Five separate 4 mL glass vials contained the following solutions:

Vial 1:1 mL of liposome

Vial 2: 1 mL of liposome, 0.240 mL of TPP-TMe-B-CDx

Vial 3: 1 mL of liposome, 0.480 mL of TPP-TMe-B-CDx

Vial 4: 1 mL of liposome, 1.195 mL of TPP-TMe-B-CDx

Vial 5: 1 mL of liposome, 2.390 mL of TPP-TMe-B-CDx

* Water was added to a final volume of 4 mL (3.0 mL – x mL of TPP-TMe-B-CDx used)*

All vials were then sonicated for 1 - $1\frac{1}{2}$ hours followed by the measurement of absorbance via UV-Vis spectroscopy. Mole ratios between TPP/Soy PC were changed from 0.00%, 0.05%, 0.10%, 0.25%, and 0.50%.

RESULTS

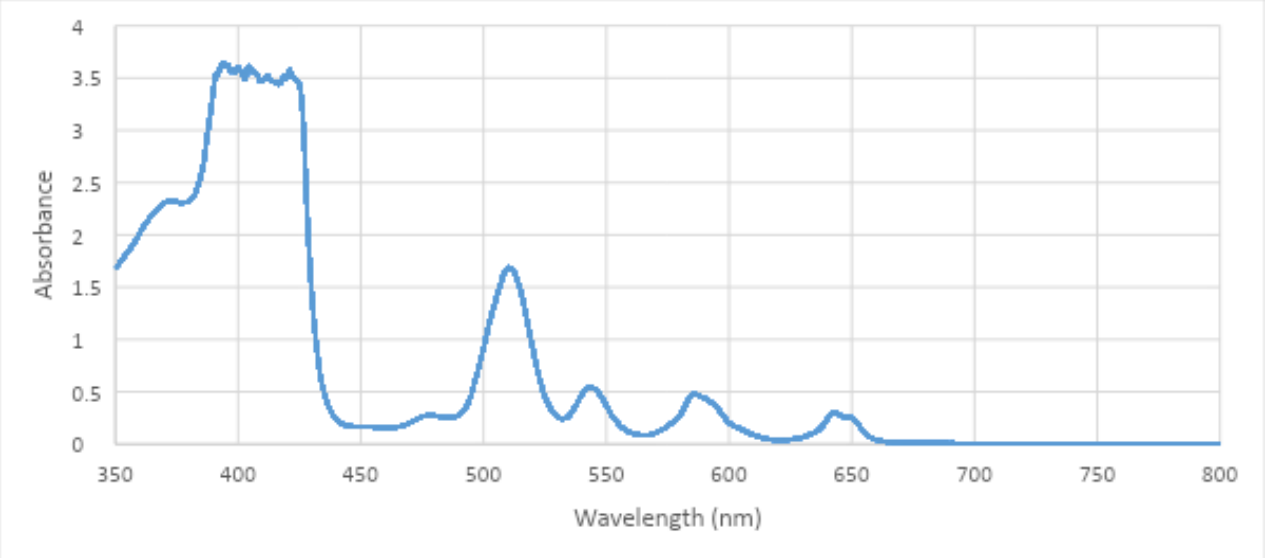

Figure 6. UV-Vis Spectra of TPP-TMe-B-CDx Complex; Confirmation indicated by absorbance peaks at 394 nm, 511 nm, 544 nm, 586 nm, and 643 nm, performed in a 1.25 cm cell.

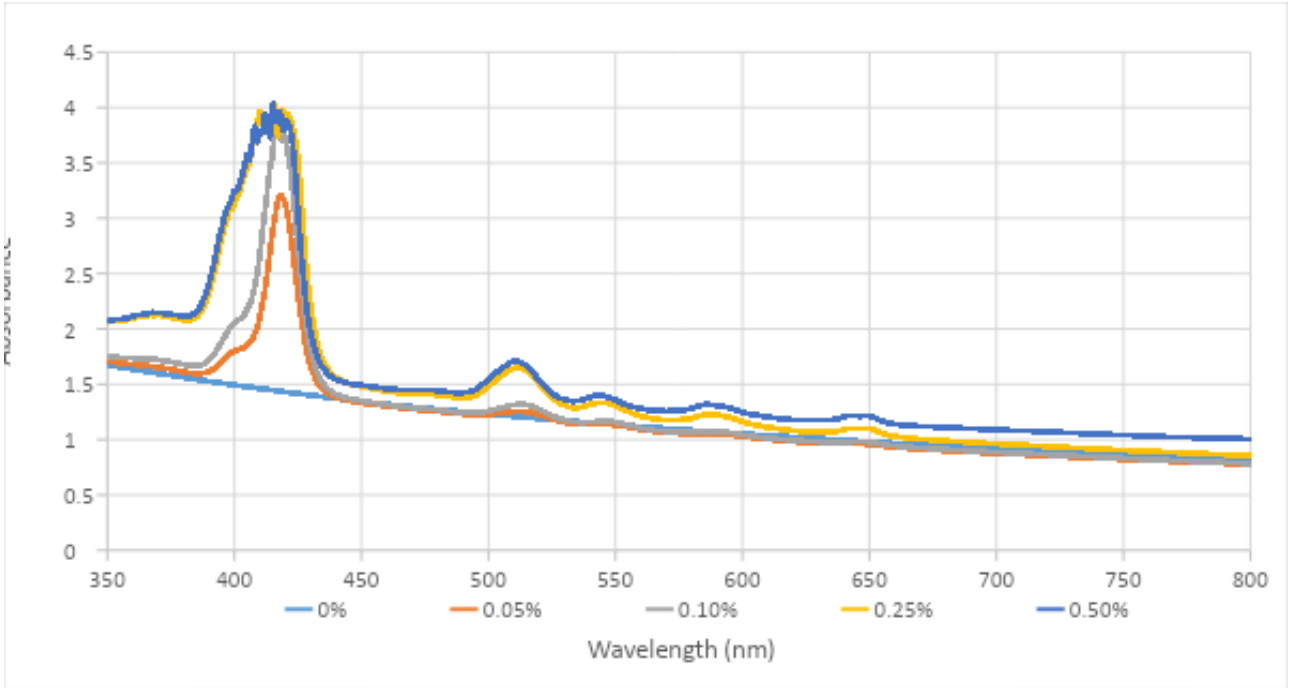

Figure 7. UV-Vis Spectra Before Sonication; Solutions of liposome & TPP-TMe-B-CDx at different mole ratios, performed in a 1.25 cm cell.

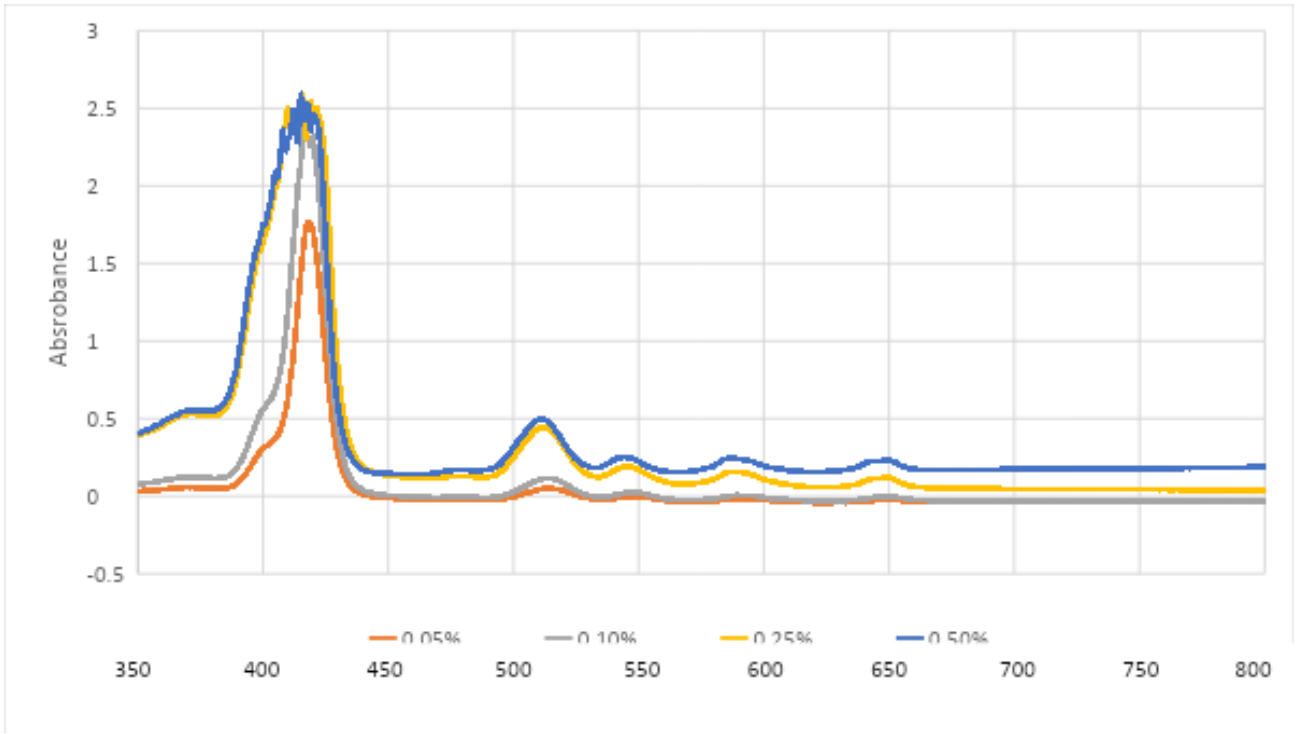

Figure 8. UV-Vis Spectra Before Sonication (Corrected Absorbance); Solution of liposome & TPP-TMe-B-CDx at different mole ratios, performed in a 1.25 cm cell. This was calculated and

plotted by subtracting the baseline absorbance values from a 0% mole ratio from each individual sample's corresponding absorbance value.

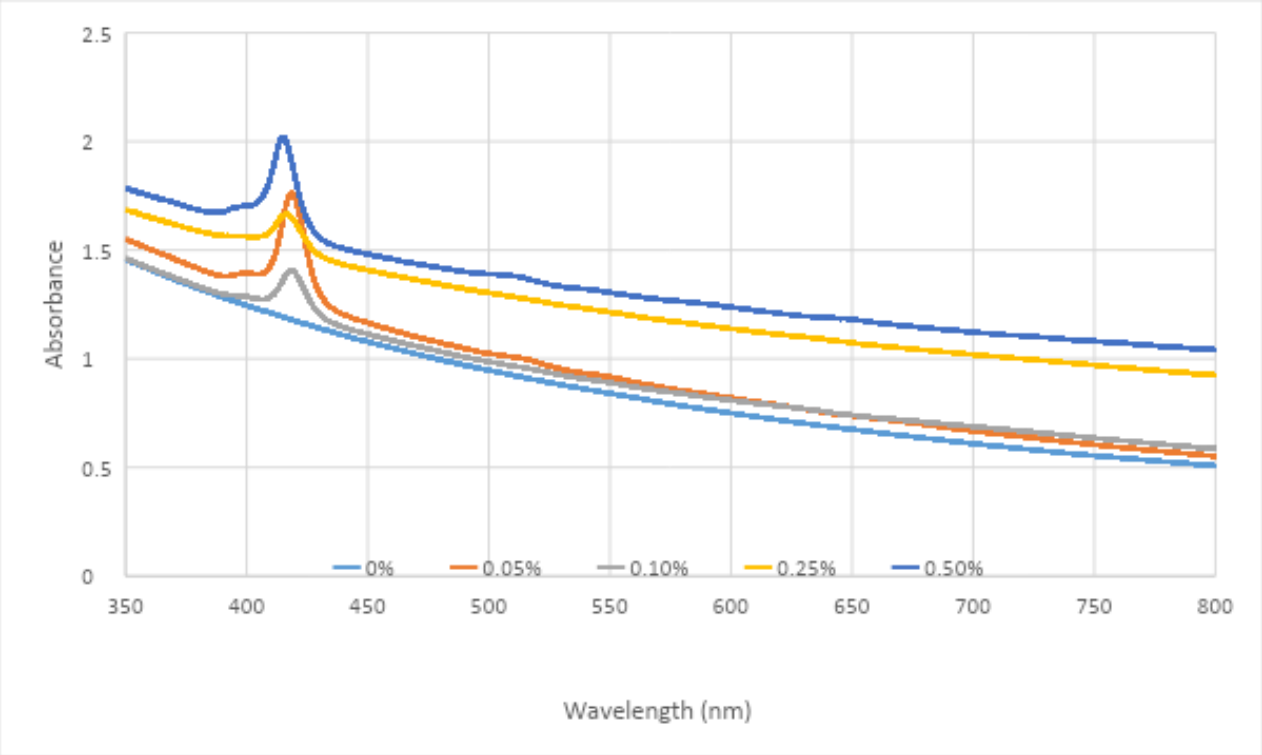

Figure 9. UV-Vis Spectra After Sonication; Solutions of liposome & TPP-TMe-B-CDx 1at different mole ratios, performed in a 1.25 cm cell.

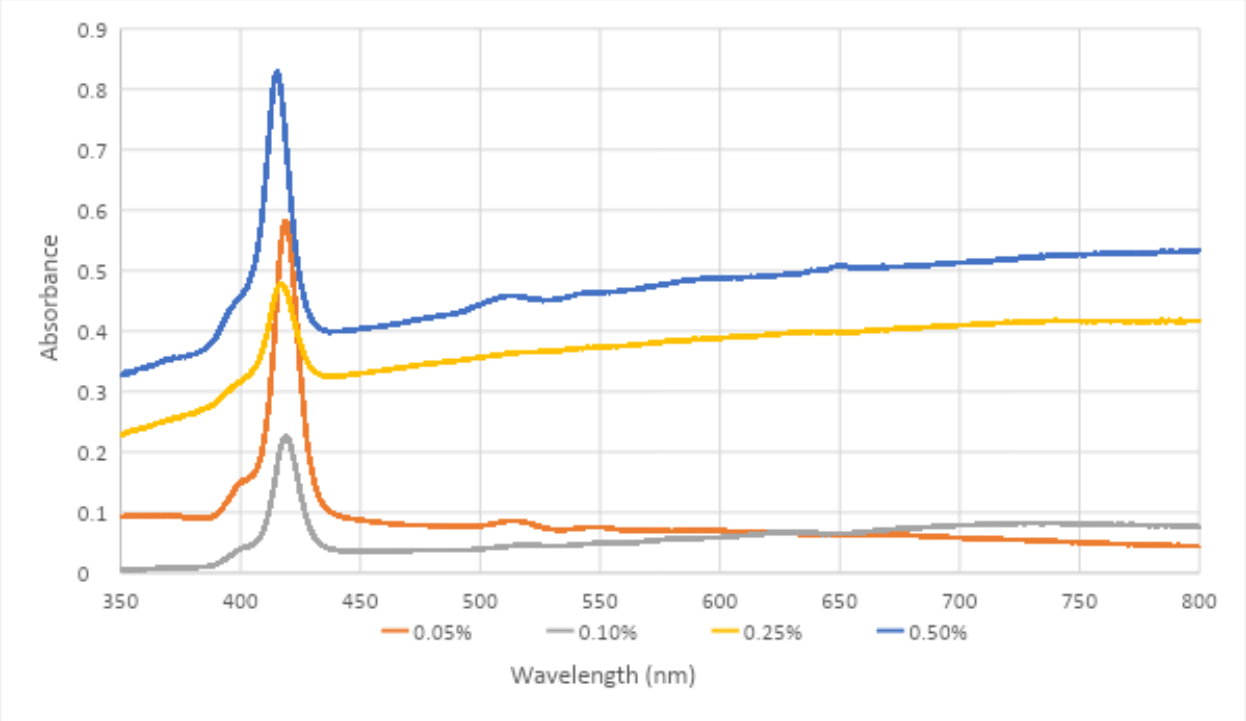

Figure 10. UV-Vis Spectra After Sonication (Corrected Absorbance); Solution of liposome & TPP-TMe-B-CDx at different mole ratios, performed in a 1.25 cm cell. This was calculated and plotted by subtracting the baseline absorbance values from a 0% mole ratio from each individual sample's corresponding absorbance value.

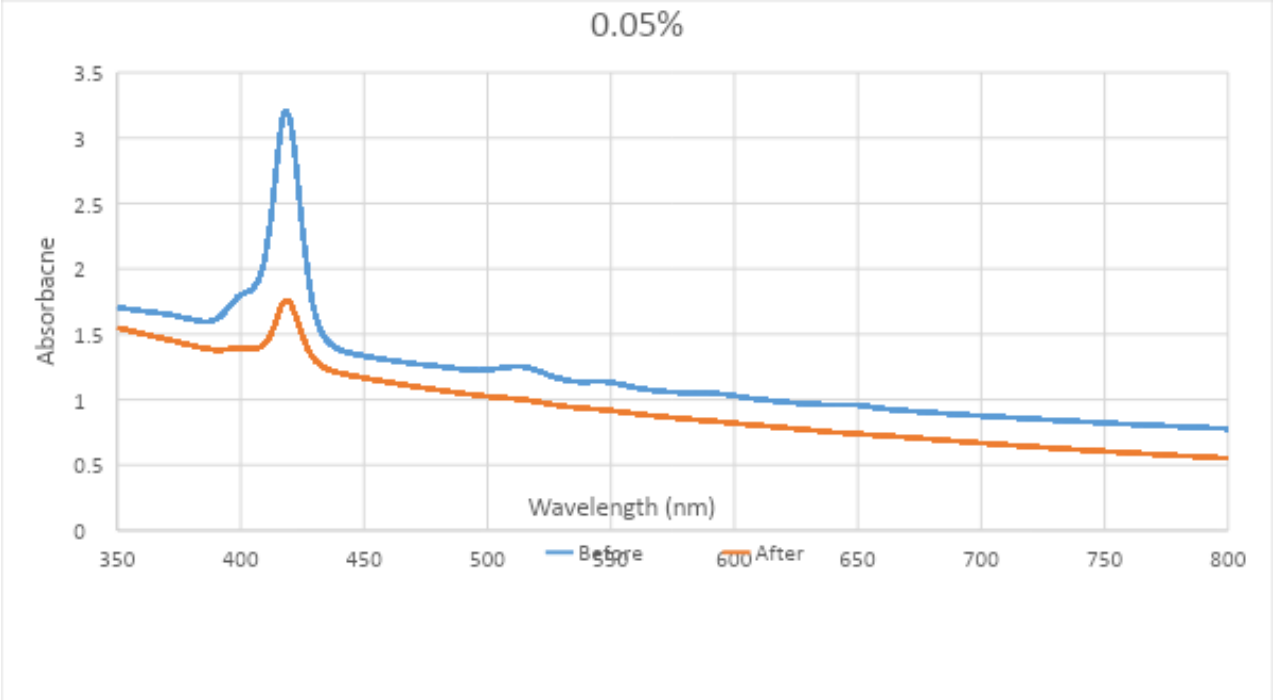

Figure 11. Comparison Data; Before & After Sonication of the 0.05% mole ratio of TPP/SOY PC.

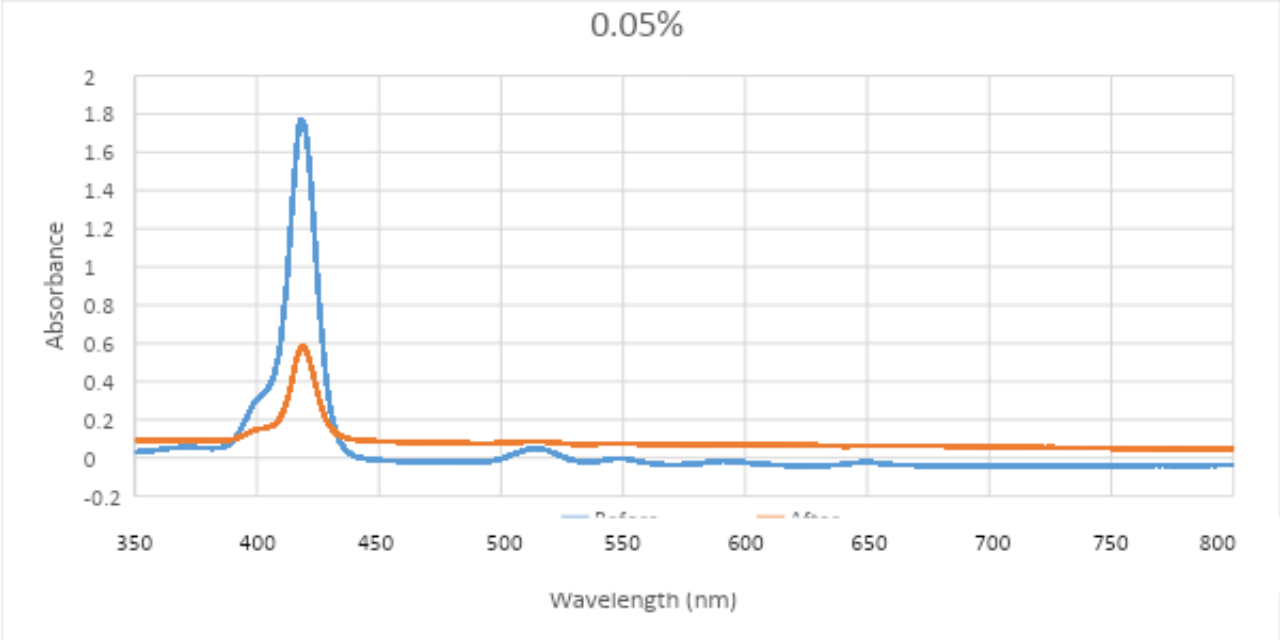

Figure 12. Comparison Data Before & After Sonication of the 0.05% mole ratio of TPP/SOY PC (Corrected Absorbance).

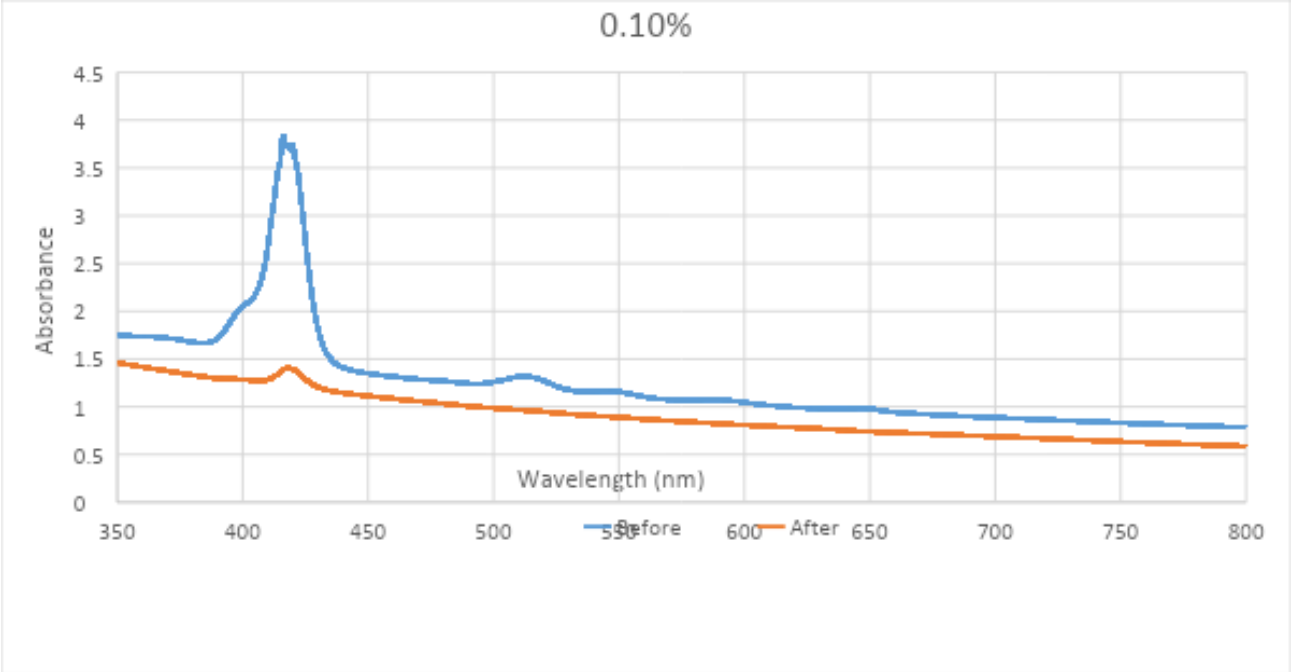

Figure 13. Comparison Data; Before & After Sonication of the 0.10% of mole ratio of TPP/SOY PC.

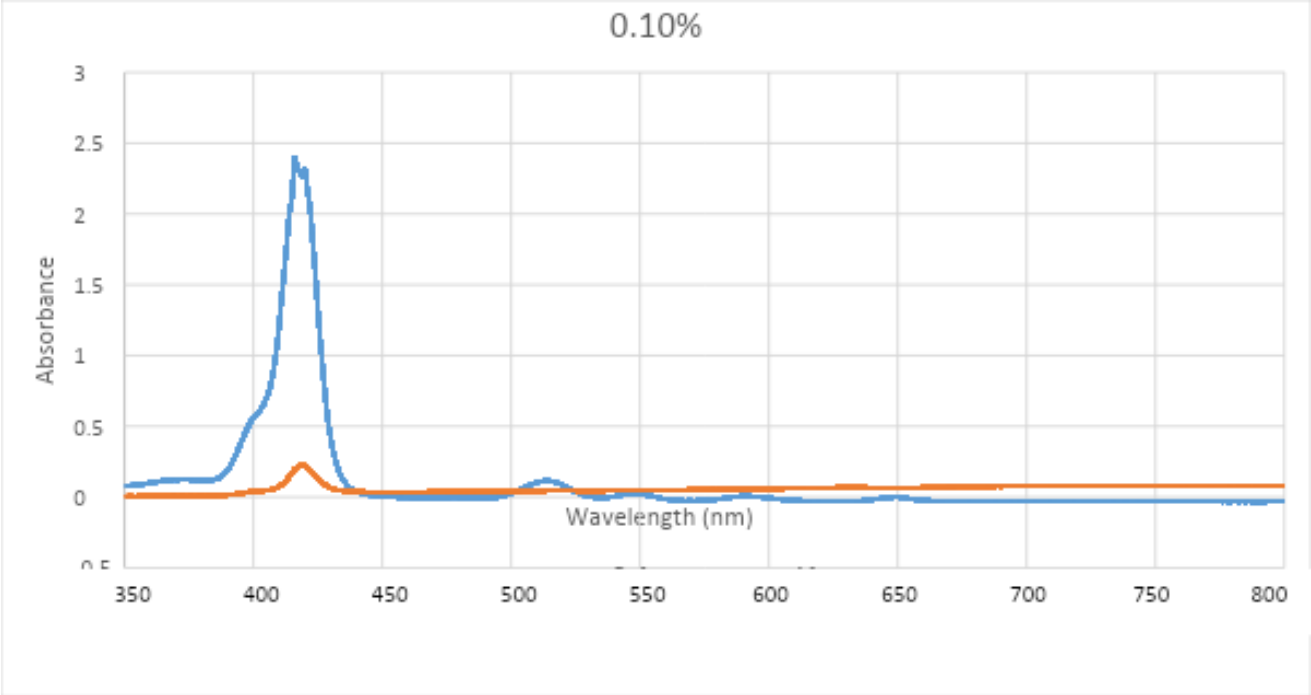

Figure 14. Comparison Data; Before & After Sonication of the 0.10% mole ratio of TPP/SOY PC (Corrected Absorbance).

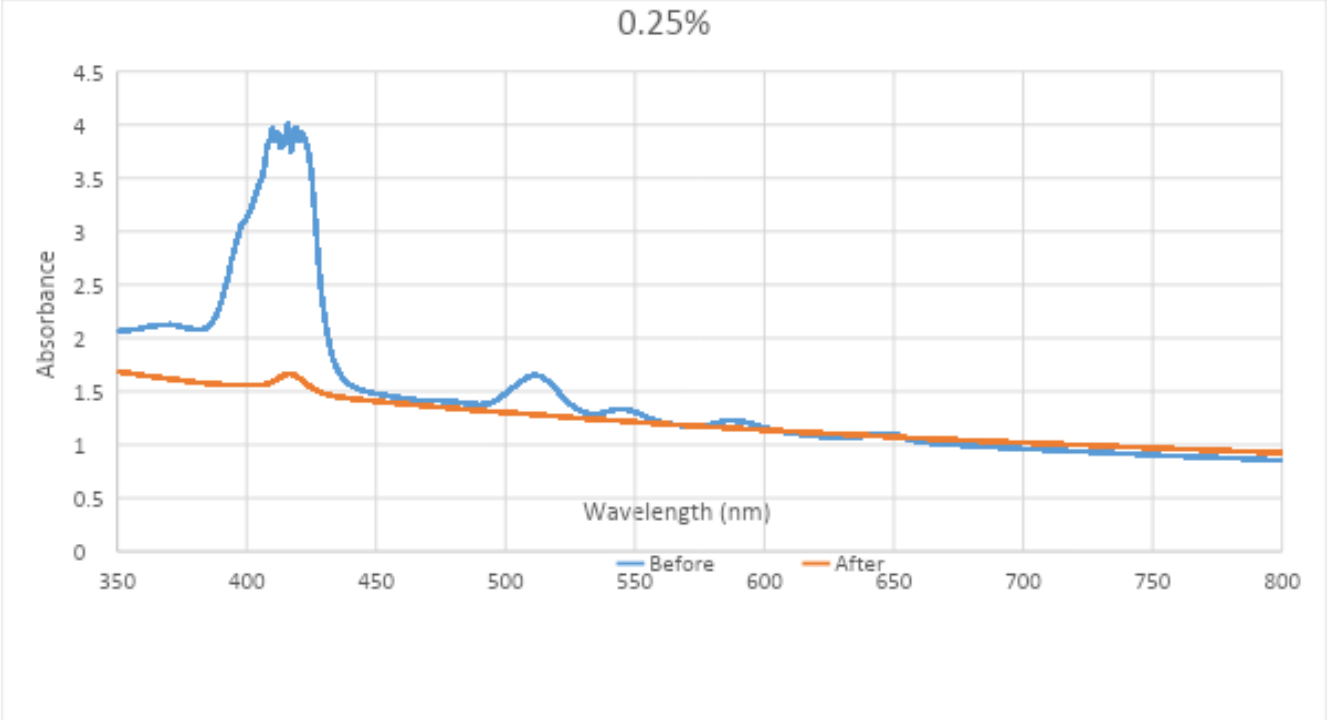

Figure 15. Comparison Data; Before & After Sonication of the 0.25% mole ratio of TPP/SOY PC.

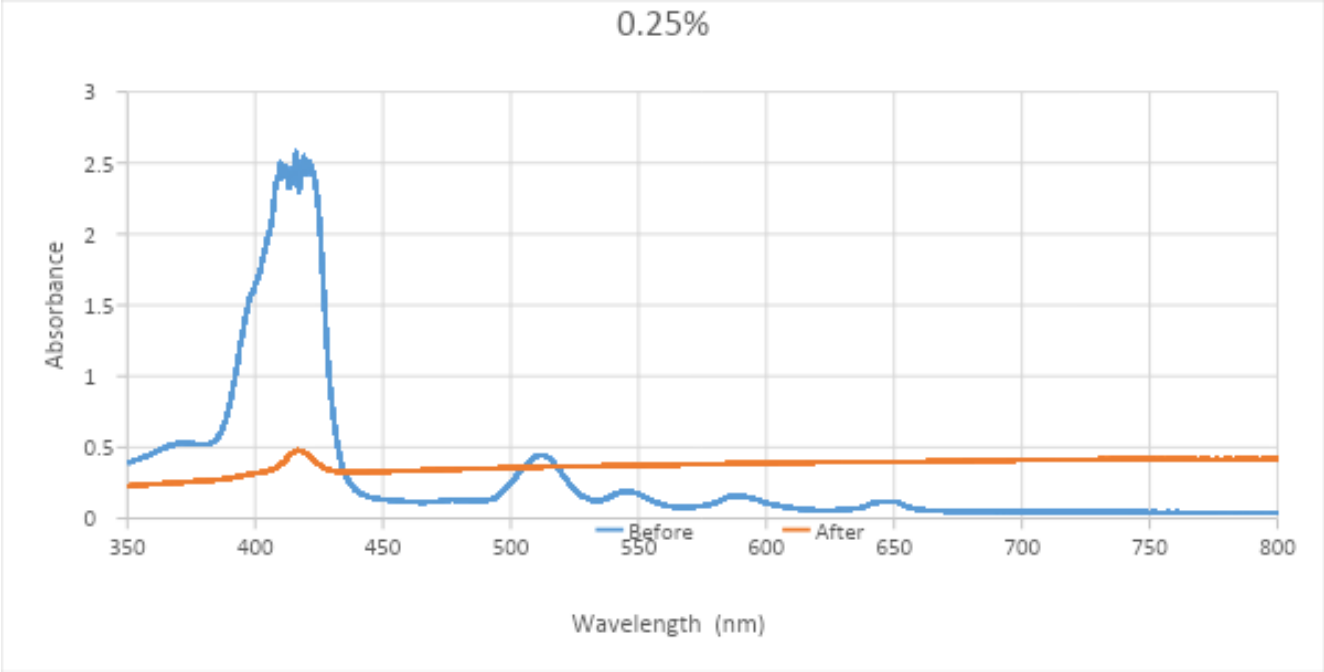

Figure 16. Comparison Data; Before & After Sonication of the 0.25% mole ratio of TPP/SOY PC (Corrected Absorbance).

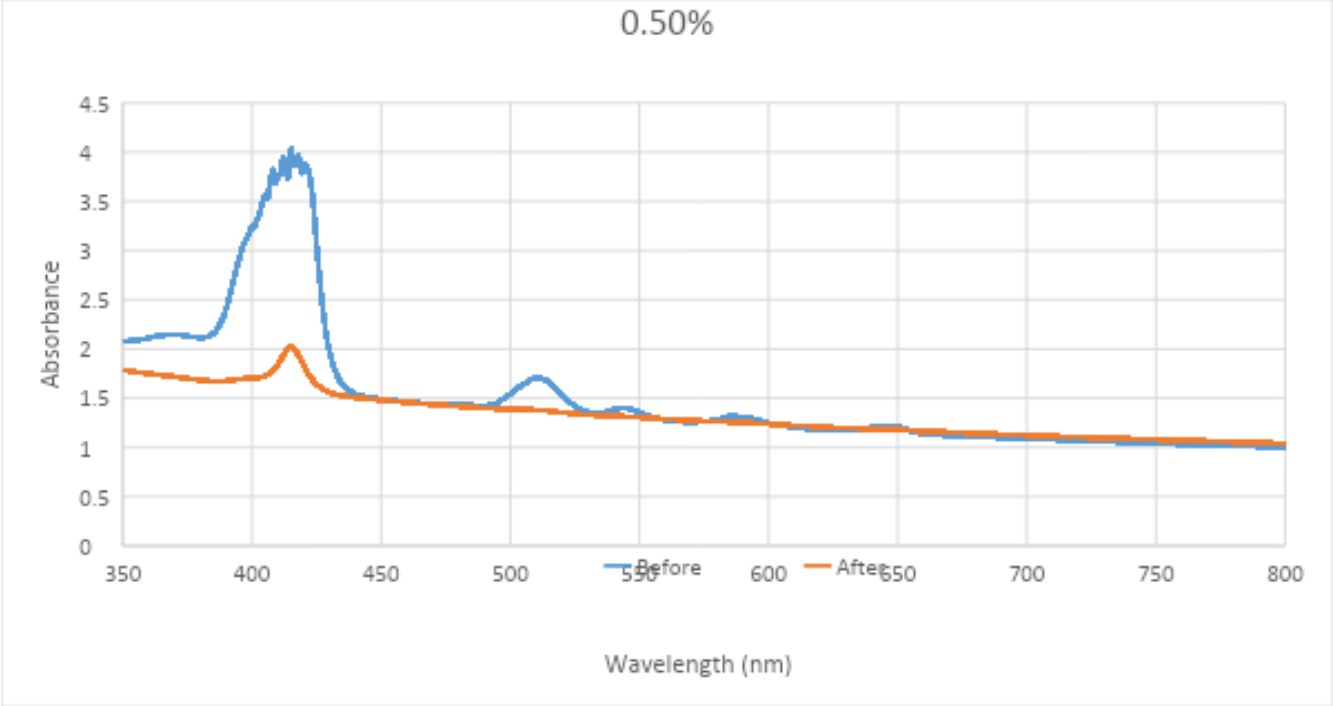

Figure 17. Comparison Data; Before & After Sonication of the 0.50% mole ratio of TPP/SOY PC.

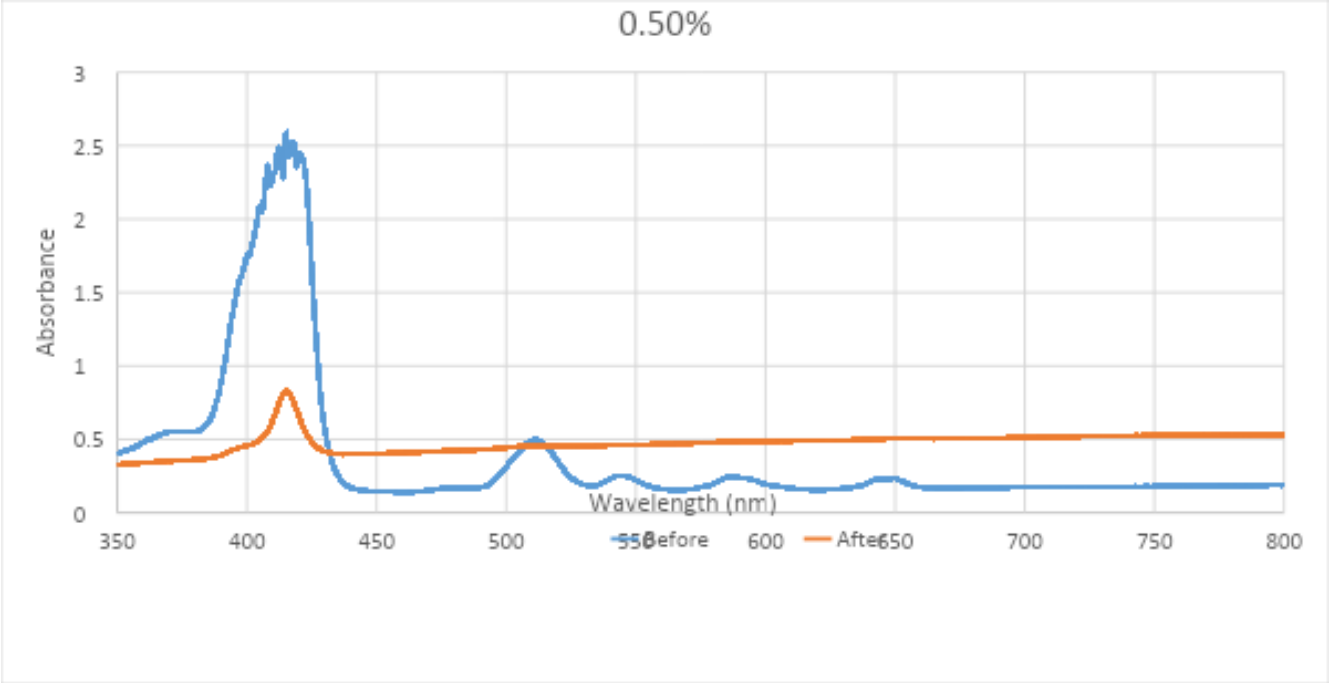

Figure 18. Comparison Data; Before & After Sonication of the 0.50% mole ratio of TPP/SOY PC (Corrected Absorbance).

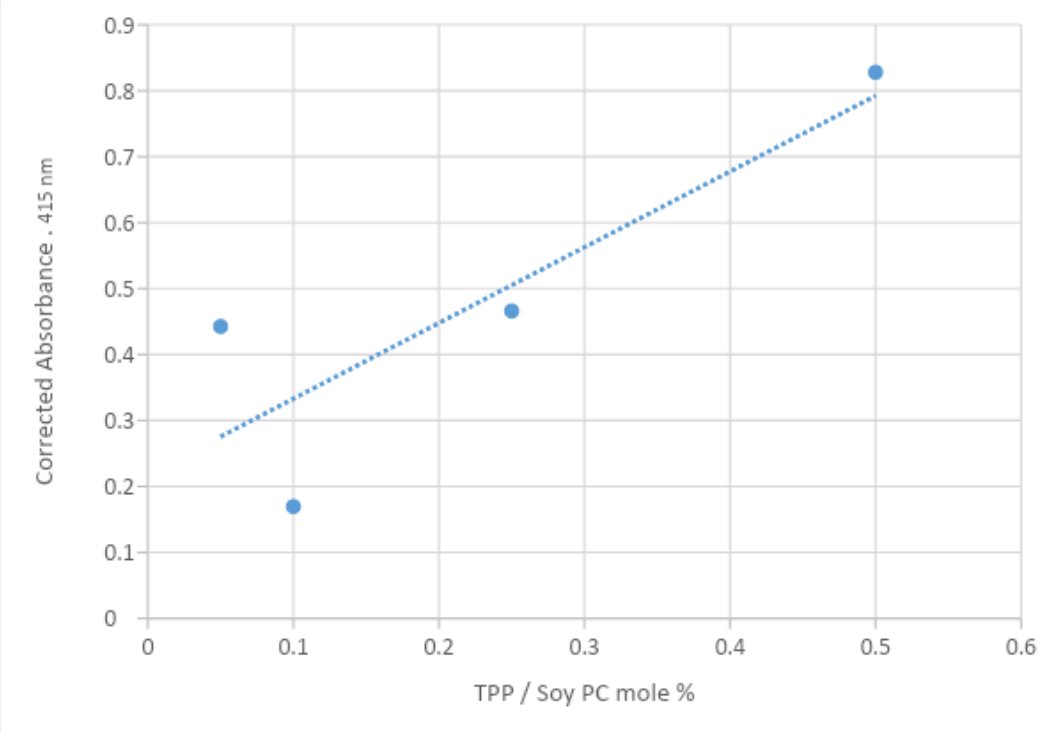

Figure 19. Abs.$_{415\ nm}$ v TPP/SOY PC mole %. As the mole ratio of TPP/SOY PC increases, the absorbance value increases linearly.

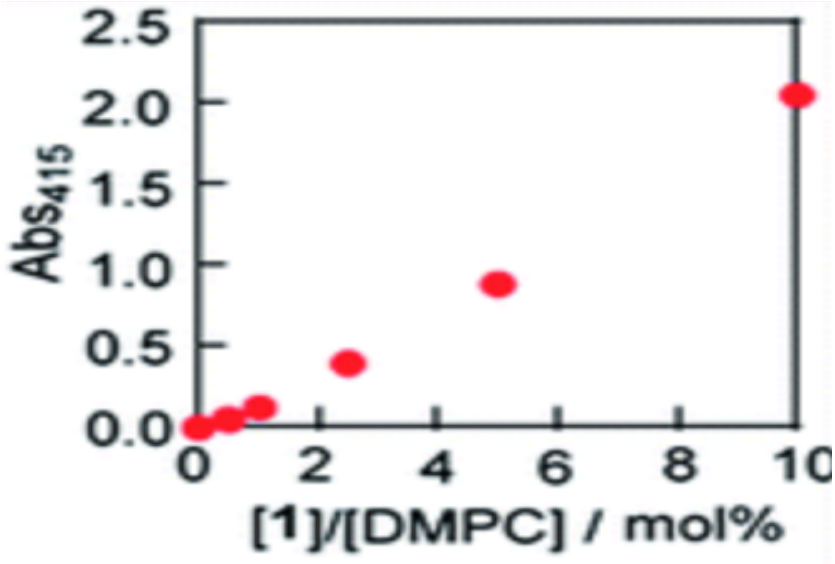

Figure 20. $Abs_{415\ nm}$ v porphyrin/liposome mole %; (mock data) [1].

DISCUSSION

The formation of the TPP-TMe-B-CDx complex was successful through observation of the purple solution, which is indicative of the proper binding of TPP within the TMe-B-CDx cavity, and the performance of a UV-Vis spectroscopy (**Figure 6**). The maximum absorbance value was 3.639905 at 394 nm (λ_{max}), corresponding to the Soret band of the porphyrin, as well as other notable wavelengths at 511 nm, 544 nm, 586 nm, 643 nm, which were associated with the Q bands. Using beer's law equation, $A = \varepsilon bc$, (in a 1.25 cm cell), the concentration of TPP was determined to be 8.377 x 10^{-3} mM.

In the preparation of loading the liposome with porphyrin, the mole ratios of TPP/Soy PC were changed from 0.00% to 0.05% to 0.10% to 0.25% and to 0.50%. Another UV-Vis spectroscopy was performed before sonication to further analyze the loading efficiency, using the 0.00% solution as a baseline (**Figure 7**). The corrected absorbance value was then calculated to account for the turbidity (cloudiness) of the liposome solution (**Figure 8**). This may influence absorbance readings to be higher than that of the TPP-TMe-B-CDx solution (**Figure 6**) when

compared to each of the mole ratio samples (**Figure 7**). This was calculated and plotted by subtracting the baseline absorbance value from each individual sample's corresponding absorbance value.

After sonication and plotting, the corrected absorbance values showed a decrease in absorbance, which suggests that porphyrin was inserted in the hydrophobic portion of the liposome (**Figures 9&10**). In the analysis of each mole ratio sample, a profound decrease in absorbance was shown, which determined that the reaction of porphyrin leaving the cavity of cyclodextrin and inserting in the liposome had occurred (**Figures 11-18**). However, the intensity of the peaks near 415 nm do not correspond to their ratios (**Figure 19**). As the mole ratio of TPP/Soy PC increases, the absorbance values after sonication should have also increased linearly (Figure 20). Liposomes can take up porphyrin as much as possible—that is, until saturation is attained; this will in turn result in a plateau of the absorbance value at some point because the remaining porphyrin is left within the solution.

CONCLUSION

The utilization of TMe-B-CDx to form a complex with TPP is a viable method for inserting high concentrations of porphyrins in liposomes. The cyclodextrin compound acts as a surfactant and decreases the water tension of TPP in order to increase solubility in aqueous solvents. Although the discrepancy between 0.05% and 0.10% absorbance values was peculiar (the higher ratio of the two was supposed to have a higher absorbance value), the liposome was still able to be loaded with porphyrin when complexed with cyclodextrin. Each ratio that was tested and observed via UV-Vis spectroscopy showed a significant decrease right after the

sonication process. These results suggest that TPP was successfully loaded into the liposome; however, not entirely, for the Soret band near 415 nm remains present.

ACKNOWLEDGEMENT

This experiment was possible due to the help of Dr. Ruomei Gao. I would like to thank her support, mentorship, and for allowing me to use her lab to conduct this project. I would also like to thank Dr. Bright Emenike for his instruction on how to conduct oneself when performing research, including how to write a research report. I would also like to thank the SUNY College at Old Westbury Chemistry & Physics Department for providing the education and facilities needed to conduct this study.

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SENIOR
SEMINAR

The Nano-Mechanical Role of Melanin in Melanoma Cells

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ABSTRACT

Melanoma is a type of skin cancer that is caused by excess exposure to ultraviolet radiation. It originates in the melanocytes of the body, as spontaneous mutation caused by UV radiation induces malignant transformation of these cells. However, current studies have shown that melanin production can affect the aggressive nature of melanoma. Scientists have found that melanin contains photoprotective properties that could affect the likelihood of being diagnosed with melanoma, depending on the concentration in the cell. They also found that the metastasis of these cells depends solely on the elasticity of the cell, ultimately leading to the discovery of the role that melanin concentration plays in taming the aggressive nature of melanoma. The goal of this paper is to analyze the nanomechanical properties of melanocytes, as well as the effect it may have on melanoma cells of different concentrations. These discoveries can improve the diagnostics protocol concerning melanoma and potentially create new treatments for patients that suffer from this disease.

INTRODUCTION

A melanocyte is a somatic cell, derived from the neural crest, that produces a dark substance called melanin, which is responsible for the pigmentation in living organisms. These cells contain a special organelle called melanosomes that produces melanin through the process of melanogenesis. Melanin can be found in various parts of the body, including the skin's epithelium and the uvea of the eye [11]. Over time, spontaneous mutation could disturb the biological pathway of the cell, causing malignant transformation [11]. One of the major parameters of this transformation is the rapid growth of these cells, which produces cancerous tumors called melanoma [11]. Known causes of melanoma include hereditary factors that might have been passed on to their offspring, the amount of exposure to sunlight over a lifetime, and personal characteristics that may affect the skin's reaction to sunlight [2].

Earlier studies on the in-depth properties of melanoma proposed that the incidence of malignant melanoma has been increasing in the Caucasian population [1]. Some of the findings indicate that melanin plays a photoprotective role as there is an inverse correlation between skin pigmentation and the incidence of sun-induced skin cancers [1]. Subjects within the "Caucasian skin" category are approximately 70 times more likely to develop skin cancer than subjects with "Black skin," suggesting that melanin has a more important role in the protection of the body [1].

Recent studies looked into the importance of melanin in different physiological and pathological processes. Some of the findings indicate that the nanomechanical role of melanin plays a huge role in the metastasis of melanoma [5]. Melanoma cells have several parameters that are required to metastasize in major organs of the body; the most important parameter is the cell's elasticity [5]. Successful melanoma metastasis requires complete invasion of the host tissue [5]. As the cells proliferate, they will encounter multiple barriers, such as the endothelial barrier. For

melanoma cells to bypass these barriers, they must undergo extensive deformation of the cell body (cell elasticity); this can be achieved by changes to the cytoskeleton during malignant transformation of the cell [5]. However, several findings also show that cells that contain melanin become stiffer, which will make it difficult for it to become deformed [5]. This will further result in the less aggressive nature of cancer due to the inhibition of one of the major parameters of the melanoma cell.

MELANIN CONTENT VS. NUMBER OF MELANOCYTES IN HUMANS

Skin color is determined by the different carotenoids, oxy-/deoxy hemoglobin, various types of melanin contained in the body, and the way the melanin is packaged and distributed by the melanosomes [8]. Without prior research, some may hypothesize that the amount of the melanocytes will depend on the ethnicity of the person; however, studies indicate otherwise [8]. Histological detection of melanin content using Fontana-Masson staining (**Figure 1.1)** has been performed on the basal layer of the skin of three different ethnicities: Caucasian, African American, and Asian skin [8]. The findings indicate that the number of melanocytes found in each skin type proves to be identical, but the melanin content in the basal layer of the epidermis conspicuously reveals that African American skin has the highest melanin content due to the significantly large size of the melanosomes, while Caucasian skin has the lowest [9,1].

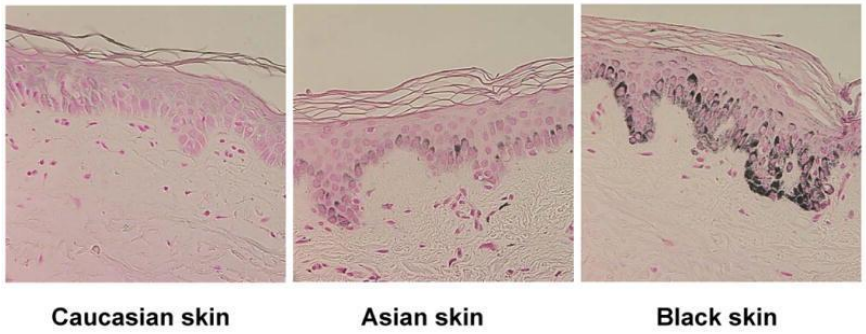

Figure 1.1- Histological detection of melanin content using Fasson- Masson staining. [1]

EFFECTS OF UV EXPOSURE IN THE PRESENCE OF MELANIN

Exposure of the skin to ultraviolet light is carcinogenic to human skin. Certain epidemiological and experimental studies have shown that melanin in the skin plays an important role, especially in photoprotection. The several studies that examined the effects of UV exposure on the skin show that the most important photo lesson that is induced by Ultraviolet B-Rays (UVB), which has the potential to cause photodamage to the skin cells' DNA, is cyclobutene pyrimidine dimers (CPD) [4,10]. With this information, several clinical studies have been used to observe the skin's reaction to UV exposure [1]. Skin samples were obtained from 90 subjects within 7 different types of ethnic backgrounds, but the emphasis of the study was on African American, Asian, and Caucasian skin [1]. Each skin sample was observed on the 1st and 7th day of the procedure after being treated with a single MED dose of UVA/UVB; African American skin, on the other hand, received 3-4 times the dosage [1]. Immunohistochemical staining was also used to identify the CPDS within the tissue samples [1]. The biopsy of the skin samples, immediately after UVR exposure, reveals that the DNA damage was significantly higher in the lighter skin tones, while significantly lower in the darker skin tones despite the extra dosage of UVA/UVB (**Figure 2.1**) [1].

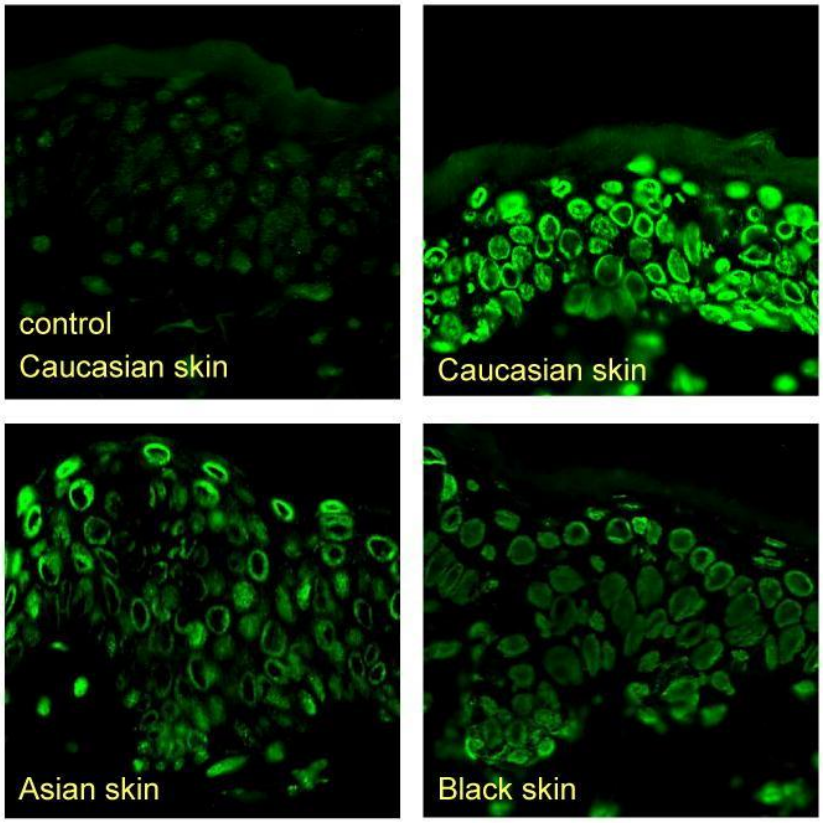

Figure 2.1 Immunohistochemical detection of CPDs in the experimental design. Fluorescence detection uses the binding of FITC-labeled antibodies that are reactive with DNA photoproducts. A higher amount of CPDs has been found in Caucasian skin compared to that of the African American or Asian skin. [1]

THE IMPORTANCE OF ELASTICITY IN MELANOMA CELLS

Regarding nanomechanical phenotypes, melanoma cells depend heavily on these properties, specifically cell elasticity, in order for them to metastasize to critical organs of the body. The primary motive of melanoma cells is to proliferate in the host tissue, and to do so, the cells must undergo a tremendous amount of deformation to get through the various barriers, especially the endothelial barrier, on the tissue [3]. This is made possible due to the malignant nature of melanoma cells, as the transformation induces the cells' cytoskeleton to turn very soft [3]. However, these studies have demonstrated that melanin granules drastically influence the elasticity properties of the cell. To prove this theory, human melanoma cells were obtained and

placed in a Ham's F10 culture medium, supplemented with 10% FBS, and kept at a constant temperature of 37℃ with a 5% CO_2 humidified atmosphere [5]. Cells were then monitored for 48 hours to make sure the synthesis of melanin was inhibited to carry on the following experiments [5].

MELANIN DETERMINATION IN CELL SAMPLES

Melanin content was determined by electron paramagnetic resonance spectroscopy, which is based on intensity and the parameters of characteristic Electron Paramagnetic Resonance (EPR) signals of eumelanin and pheomelanin [5]. The cells that were incubated for 48 hours were detached from their culture, pelleted, counted, and incubated in high concentrations of zinc ions (50 mM) [5]. EPR examination was carried out in liquid nitrogen, using standard finger-type quartz, and EMX-AA spectrometer, operating at X-band with 100 kHz magnetic modulation [5]. The average number of melanin granules was also found by comparing double integrals to synthetic melanin used as a standard, which is found to be about 2 x 10^8 granules. **Figure 3.1** shows the EPR examination among all the tested samples of cells [5].

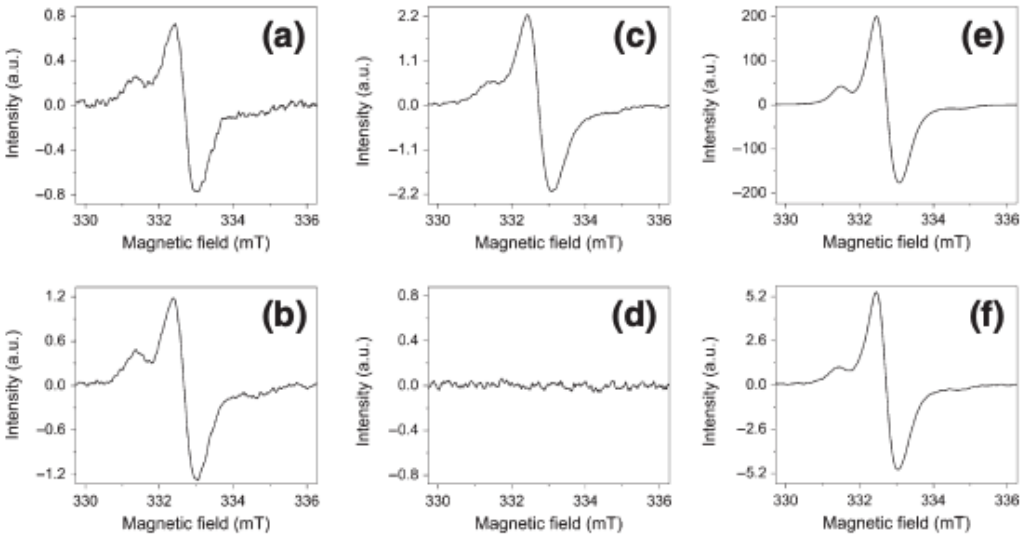

Figure 3.1 The determination of melanin content inside the cells. (**A-C**) Represents the spectra of melanoma cells with melanin contents. (**D**) Represents a non-pigmented cell, serving as a control. (**E**) Represents the synthetic cysteine- L dopa melanin. Lastly, (**F**) represents purified melanin granules isolated that worked as a standard in determining the average number of melanin granules per cell sample. [5]

ELASTICITY OF THE DIFFERENT MELANOMA CELLS

Mechanical analysis of the cell's elasticity in these samples was conducted using Agilent 5500 Atomic Force microscopy [5]. Cells were maintained at a median of 37℃. The measurements of the AFM were produced using silicon nitride cantilevers, with a nominal tip radius at 20 nm [5]. 40 cells of each of the three cultures were tested in the AFM, while the fourth culture was used as a control group [5]. The pressure was placed on the cell while the measurements of the force-displacement curves were collected [5]. These curves were recorded at a rate of 1 Hz per curve from a grid of 8x8 curves and were then applied onto the central area of the cells [5]. Before the grid was selected, a topography image of the cell was obtained to make sure that the grid was used at the center of the cell [5]. As mechanical measurements were being taken, the force of the AFM was carefully monitored to avoid induced effects and damage to the cell [5]. To also gather

information on the colocalization effect on the melanin granules of the cell, representative force maps were produced on the pigmented and non-pigmented cells [5].

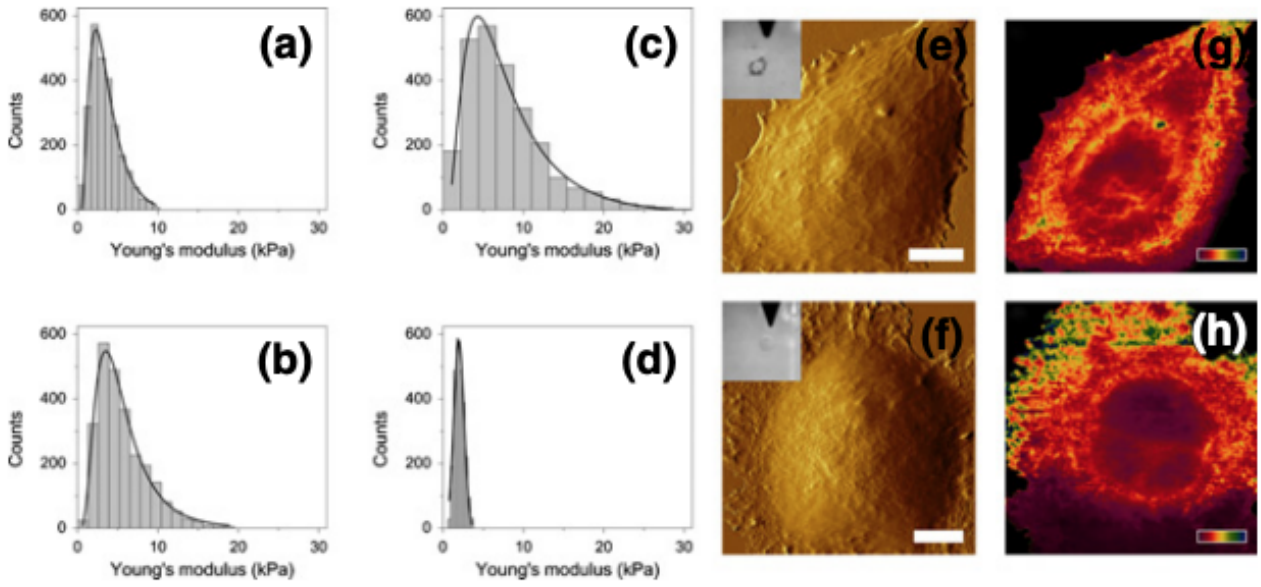

Figure 3.2 The influence of melanin granules on the elasticity of melanoma cells. A Young's modulus histogram has been developed showing the values for cells with a different number of melanosomes: (**A**) contains 22 plus/minus 2 (the 2 in regards to the standard deviation) melanosomes, (**B**) contains 42 plus/minus 3, (**C**) contains 73 plus/minus 4, and (**D**) contains no melanosomes, as its being used as a control in the case. 40 cells have been analyzed from each well, as the 64 measuring points on each cell. (**E-H**) are the representative images of the cells that underwent the AFM. (**E, F**) represents the amplitude images of the pigmented and non-pigmented cells, respectively. [5]

The histograms on the left-hand side of **Figure 3.2** show the values that were received from the AFM. According to the data, the most pigmented cells (**C**) shows the highest values in the Young's modulus containing the 73 melanosomes, (**B**) comes in second containing 42 melanosomes, and (**C**) shows the lowest values in the Young modulus containing 22 melanosomes. The images on the right-hand side of **Figure 3.2** show the representative images of the (**E, G**) pigmented cells that maintain their cytoskeletal integrity and (**F, H**) non-pigmented cells that become deformed.

METASTATIC TEST OF MELANOMA CELLS

Several tests were conducted to see the metastatic properties of the melanoma cells under the influence of various melanin concentrations. Firstly, the transmigration efficiency of the cell was determined by the Transwell assay to analyze the invasive properties of the cells [5]. To ensure that the assay highlighted the mechanical aspects of the cells, the membrane was not coated with collagens or Matrigel [5]. These cells were seeded into 6 wells (3 wells per condition); then after 24 hours, the cells were trypsinized and the number of cells that transmigrated through the porous membrane were counted [5].

Secondly, the proliferation of the cells was analyzed. The cells were separated into 12 wells (1 condition per well) and maintained in each culture for 96 hours (4 days). Every 24 hours the cells were trypsinized and counted [5].

Thirdly, the migration activity was observed based on time-lapse monitoring of the motion in each cell, which was conducted by using a fully automated Lecia DMI6000 inverted microscope with an attached incubation chamber [5]. These cells were maintained in the cell culture medium at 37℃ in a humidified environment of 5% CO_2 while being recorded for 8 hours in 5-minute intervals [5].

A fourth assessment used gelatin zymography assay to see if the melanoma cells express the pro-MMP-9 protein. Before the analysis, the cells were incubated in a serum-free medium for 24 hours [5]. Afterward, the supernatant was collected to extract similar amounts of protein from each sample and diluted in a sample buffer [5]. Each sample underwent electrophoresis on SDS-PAGE gels containing 1% gelatin [5]. After electrophoresis, gels were washed in 2.5% Triton X-100 for 30 min to remove SDS [5]. They were then placed in a substrate buffer and incubated for 40 hours under gentle agitation [5]. After incubation, gels were stained with the following: 40%

methanol, 7% glacial acetic acid solution containing 0.4% Coomassie Brilliant Blue for 45 minutes, and 7% glacial acetic acid solution (without 0.4% Coomassie Brilliant Blue) for another 30 minutes [5]. All these assays were performed three times to improve the validity of the experiment.

Lastly, an immunofluorescent image was formed on the cell as they were fixed with 3.7% formaldehyde, permeabilized with 0.1% Triton X-100, blocked with 3% BSA, and stained with mouse monoclonal antibody against human microtubule [5]. This was followed by Alexa Fluor 488goat anti-mouse IgG, with TRITC-phalloidin and then Hoechst 33342 (Sigma-Aldrich), respectively [5]. Images were taken with a Nikon Ti-E A1 inverted microscope coupled with a laser scanning module equipped with: 405, 488, 561, and 638 nm laser diodes, 4 PMT detectors for fluorescence, and 1 PMT detector for transmission [5]. Pictures of the cells were analyzed by Nis- Elements AR 3.1 software.

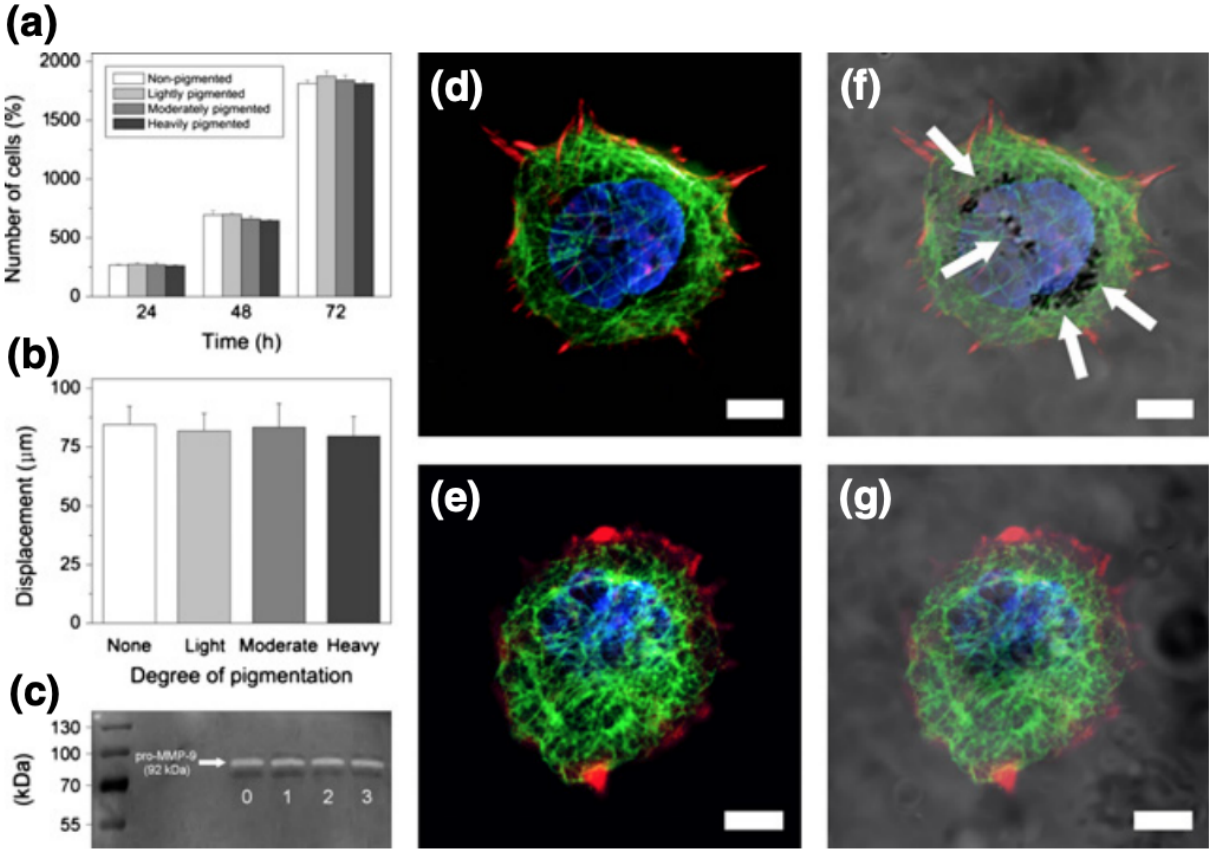

Figure 3.3 Shows the impact that melanin granules have on melanoma cell's vital functions and the cytoskeleton. (**A**) shows a graph of proliferation abilities of pigmented and non-pigmented melanoma cells, with information obtained from three independent experiments. (**B**) is a graph representing the migration activity of the pigmented and non-pigmented cells based on the motion of the individual cells. (**C**) shows the gel electrophoresis from the three zymographies that were taken to identify the expression of pro-MMP-9 for pigmented and non-pigmented cells. A list of the following was represented: 0-non-pigmented, 1-Lightly pigmented, 2- moderate pigmented, 3-heavily pigmented. (**D-G**) are images of the confocal microscopy. The color in the images represents the following: (Red) Actin, (Green) Microtubules, and (Blue) Nucleolus. (**D, E**) represents pigmented and non-pigmented cells, while the images on the right-hand side of (**D, E**) are the same; however, the images on the right labeled the melanin granules found in the cell. No melanin granules are present in (**G**). [5]

The data in the graphs (**A, B**) indicate that the proliferation and migration activity in the cell seem similar between each trial, which expresses that neither proliferation nor migration activity has been inhibited by the melanin granules. A similar outcome was found in (**C**) as it shows that each of the samples expressed pro-MMP-9. Lastly, the fluorescent images indicate that melanin granules do not affect the cells' cytoskeletal integrity.

Degree of cell pigmentation	Number of melanin granules[1]	Young's modulus (kPa)[2]	Transmigration efficiency (%)[3]
Non-pigmented	–	1.97 ± 0.05	18.79 ± 0.51
Lightly pigmented	22 ± 2	3.23 ± 0.85*	13.83 ± 0.76*
Moderately pigmented	42 ± 3	4.91 ± 1.22**	9.18 ± 0.46**
Heavily pigmented	73 ± 4	6.97 ± 1.56***	3.94 ± 0.27***

Table 1. represent the statistical values of the average number of melanin granules per cell, average values of Young modulus (elastic deformity), and the transmigration efficiency of the cells. [5]

The data shown in **Table 1** indicate a correlation between the number of melanin granules and Young modulus/Transmigration efficiency. The Young modulus values indicate that as the average number of melanin granules increases by sample, the cells are able to withstand more kPa^2. The transmigration efficiency column also indicates that it will decrease as the melanin granules increase.

Influence Of Endogenous Melanin On The Nanomechanical Phenotype Of Melanoma Cells

Even though most cancer cells originate from a single malignant cell, spontaneous mutations during the progression of the cancer cells create differences between individual cells [6]. As a result, only a small portion of cells acquire metastatic properties. Searches have been done to distinguish the metastatic and non-metastatic cells, as such markers would lead to better diagnosis of patients. Recent studies between cellular elasticity and melanoma cells have shown to exhibit invasive potential [6]. Based on prior observations, nanomechanical phenotypes of cancer cells are viewed to be a major indicator in metastatic melanoma and have the potential to be a diagnostic marker of cancer [6].

The nanomechanical influences of melanin on melanoma cells were ignored for a long time. It was believed that the unusual nature of melanosomes was the reason why pigmented melanoma cells became very stiff and difficult to deform [6]. However, recent studies of these unusual properties revealed that the presence of induced melanin was rather the cause of stiffness and subsequent deformation difficulty in melanoma cells [6]. This current work takes into account the natural or endogenous pigment concerning the mechanical effect it has on melanoma cells [6]. In this work, samples from Bomirski hamster melanoma cells with endogenous pigment have been isolated from hamster tumors to observe the nanomechanical role of melanin in its purest state [6].

Melanin Determination In Endogenous Melanoma Cells

To determine the melanin concentration of the cell samples that were taken, an EPR was conducted (similar protocol that was used in the previous paper was implemented) [5]. The

experimental design consists of 6 groups where the melanin concentration was decreased respectively (**Figure 4.1**): BHM Ma P1, BHM Ma P2, BHM Ma P3, BHM Ma P4, BHM Ma P5, and BHM Ab (amelanotic) [5].

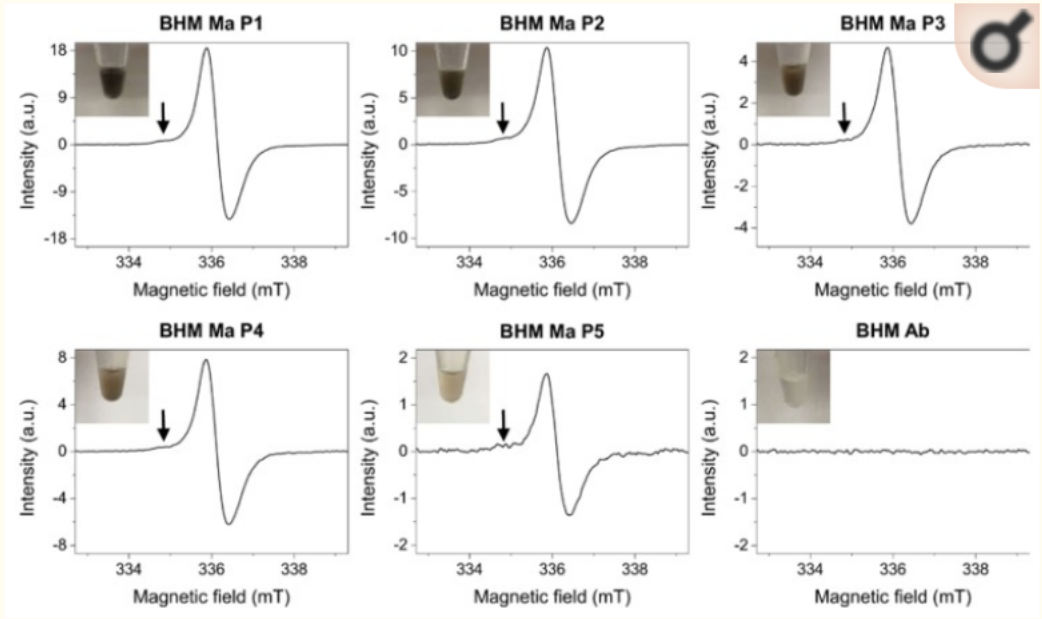

Figure 4.1 represents the Electron Paramagnetic Resonance (EPR) spectra of the melanoma cells being examined. The arrow indicates the lower field component that is allocated to the pheomelanin pigment. [5]

PROLIFERATION IN BHM CELLS

The proliferation of the cells was also taken into account in these protocols. **Figure 4.2** shows the proliferation of the cells of the various groups that were examined in this protocol. In these graphs, it's evident that the heavier pigmented cells display slower proliferation [6]. Stability became established between samples as the melanin content in each cell decreased [6]. BHM Ab cells had the most stable graph, while the BHM Ma P1 cells had less correlation between the proliferation of cells and time in hours [6].

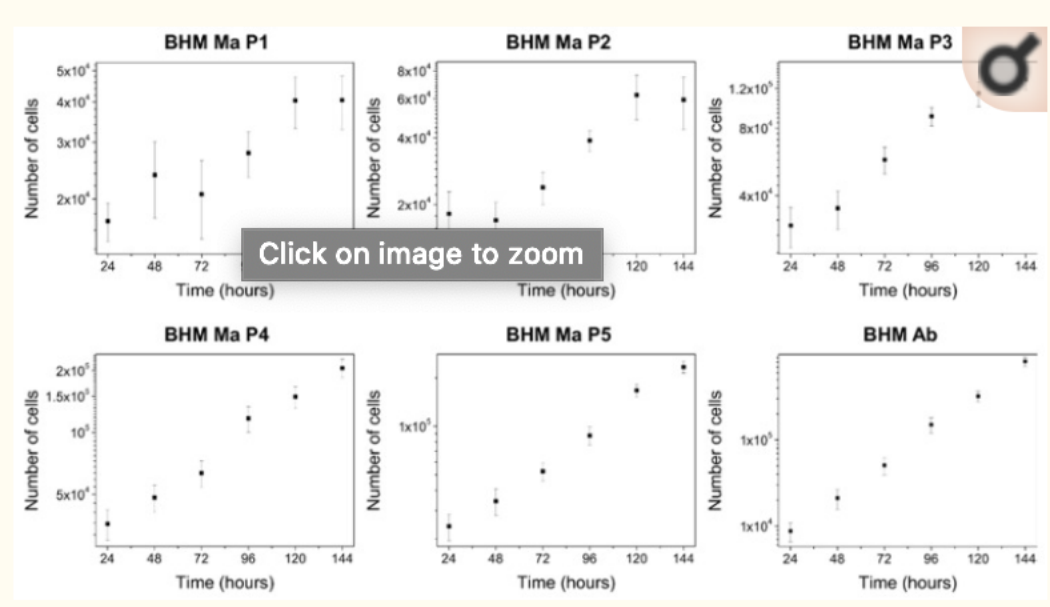

Figure 4.2 represents the growth curves of the BHM cells on a logarithmic scale. [6]

CYTOSKELETAL ANALYSIS

The organization of the cytoskeleton was examined using laser scanning confocal microscopy (LSCM) for analysis. **Figure 4.3** shows the imagery that was portrayed by LSCM. The more pigmented cells (BHM Ma P1) have a rounder morphology, while the cells that follow show a triangular morphology [6]. The fluorescent images also show that the BHM Ma P1 cells had less proliferation compared to the other cell samples in the experiment [6].

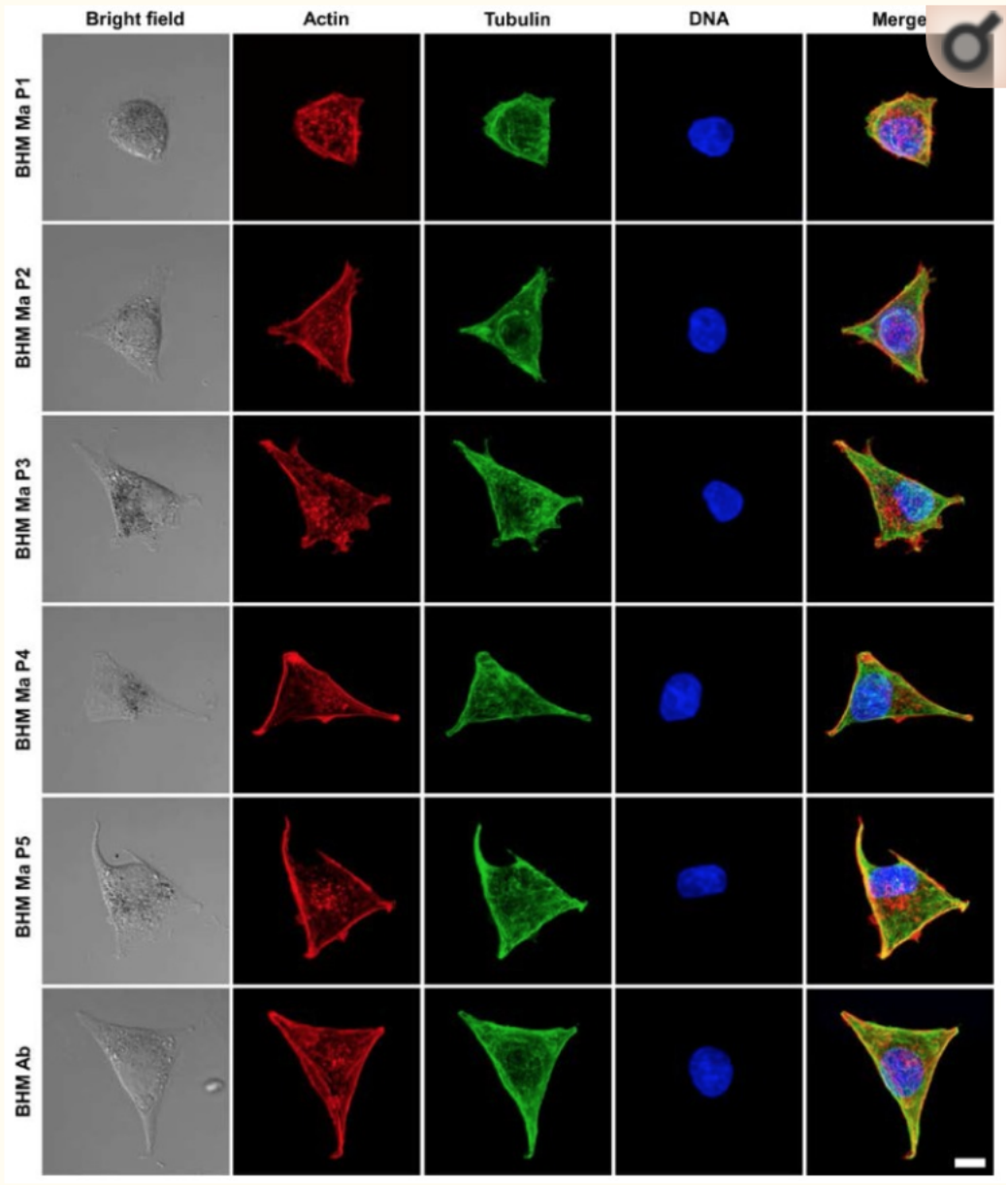

Figure 4.3 shows the LSCM images that were examined. The first column shows the morphology of the cells. The images on the right show the fluorescent images of these cells, representing the different organelles that compose these cells. [6]

NANOMECHANICAL ROLE OF MELANIN IN BHM CELLS

Lastly, the nanomechanical properties of these cells were examined using atomic force spectroscopy (AFS). **Figure 4.4** indicates that the values of the Young's modulus decreased

between samples. BHM Ma P1 shows to have the highest values and BHM Ab shows to have the lowest values due to being amelanotic [6].

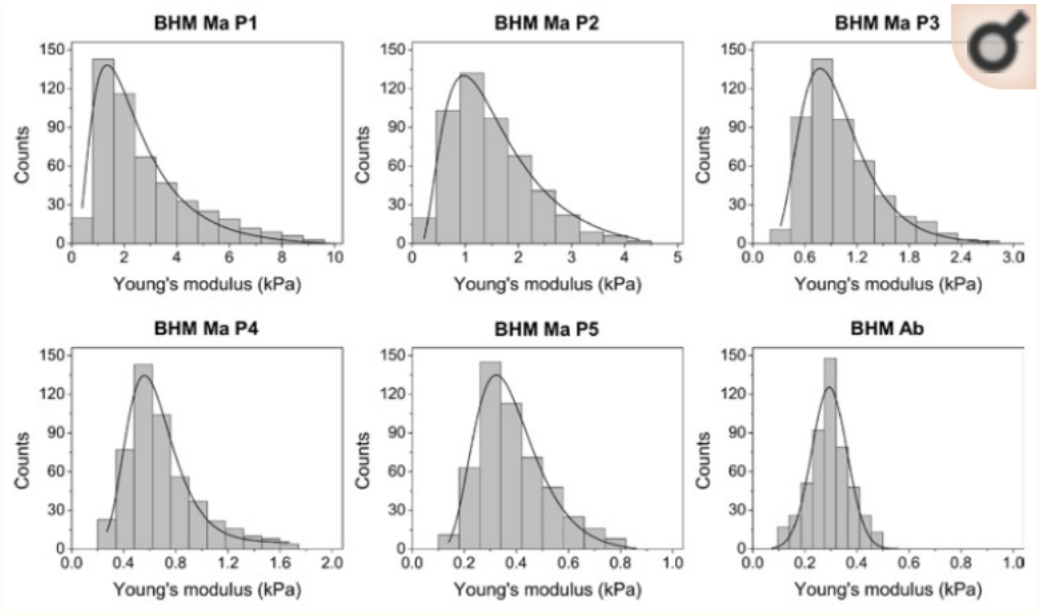

Figure 4.4 Represents the histograms of the Young modulus values that were recorded from the experiment. The solid line was present to fit the function of data. [6]

Cell Sample	Melanin Content (ng/Cell)	Doubling Time (h)	Young's Modulus (kPa)
BHM Ma P1	0.24 ± 0.02	126.8 ± 20.3	2.27 ± 0.19
BHM Ma P2	0.13 ± 0.01	76.2 ± 15.2	1.46 ± 0.13 *
BHM Ma P3	0.081 ± 0.007	63.3 ± 11.8	0.89 ± 0.09 **
BHM Ma P4	0.038 ± 0.005	57.9 ± 7.1	0.63 ± 0.07 ***
BHM Ma P5	0.019 ± 0.003	43.9 ± 3.7	0.39 ± 0.05 ****
BHM Ab	-	22.1 ± 1.2	0.28 ± 0.01 *****

Table 2 shows the numerical values of figures 4.1, 4.2, and 4.4. [6]

PRESENCE OF MELANIN INHIBITS MELANOMA CELLS

Prior studies have focused on the nanomechanical roles of melanin and cell elasticity in the spread of melanoma cells on the host tissue. However, this study observes how the presence of melanin affects the ability of melanoma cells to proliferate and metastasize. Inoculated human melanoma cells with different melanin content were injected into nude mice to examine the nanomechanical phenotype, and shed new light on the newly found effect that melanin has on the metastasis of melanoma [6].

MELANIN DETERMINATION

Once again, the presence of melanin was tested between three cell samples using an EPR. **Figure 5.1** shows three separate cell samples that were induced with different amounts of melanin due to the medium used to culture the cells.

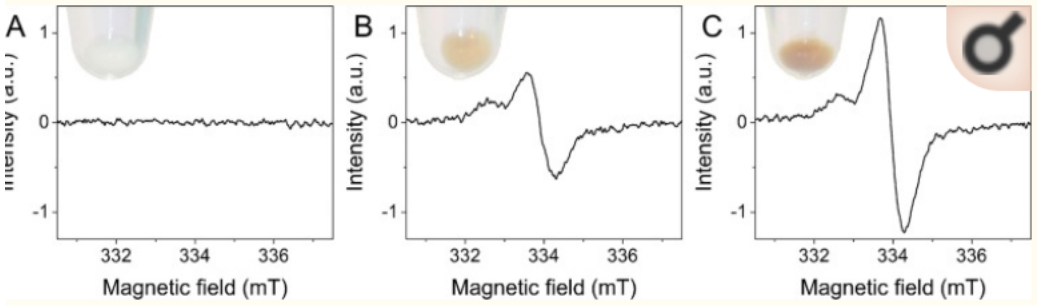

Figure 5.1 shows the Melanin determination of the cells that are being used in the experiment. (**A**) represents the non-pigmented cells, (**B**) shows moderately pigmented cells, and (**C**) represents heavily pigmented cells. The images in the left-hand corner of each graph show the cell pellet used in the EPR. [5]

YOUNG'S MODULUS OF MELANOMA CELLS

Nanomechanical properties of these cells were examined using Young's modulus to determine the stiffness of the cell, as well as the elastic deformity (**Figure 5.2**) The graph indicated that the Young's modulus values increased as the cell became heavily pigmented. Regarding elastic deformation, the values decreased as the melanin content increased [5].

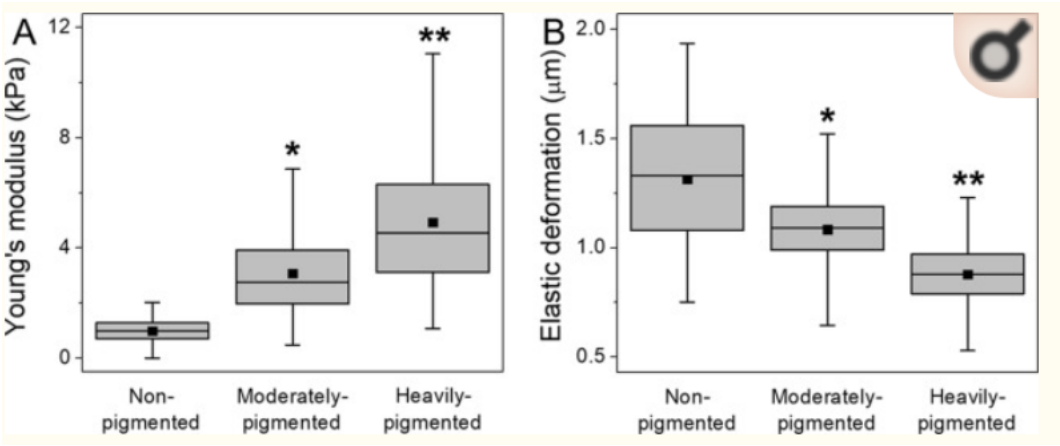

Figure 5.2 shows the values of Young's modulus (**A**), as well as the measured elastic deformation (**B**) of these cells in a box plot. [5]

MASS OF MICE LIVERS THAT CONTAIN TUMORS

The number of tumors and mass of the livers were examined during the autopsy of the mice (**Figure 5.3 & 5.4)**. The results of the autopsy indicates that the mass of the livers, as well as the number of tumors dramatically decrease as the pigment in the cell increases [5].

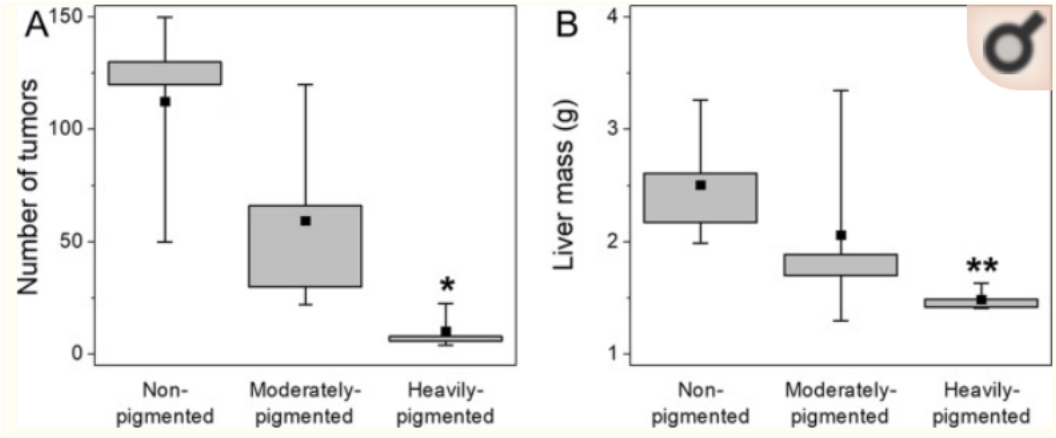

Figure 5.3 Expresses the results from the examination of the mice livers in a box plot. (**A**) represents the number of tumors found in the liver, which contained the inoculated melanoma cells. (**B**) represents the mass of the liver. [5]

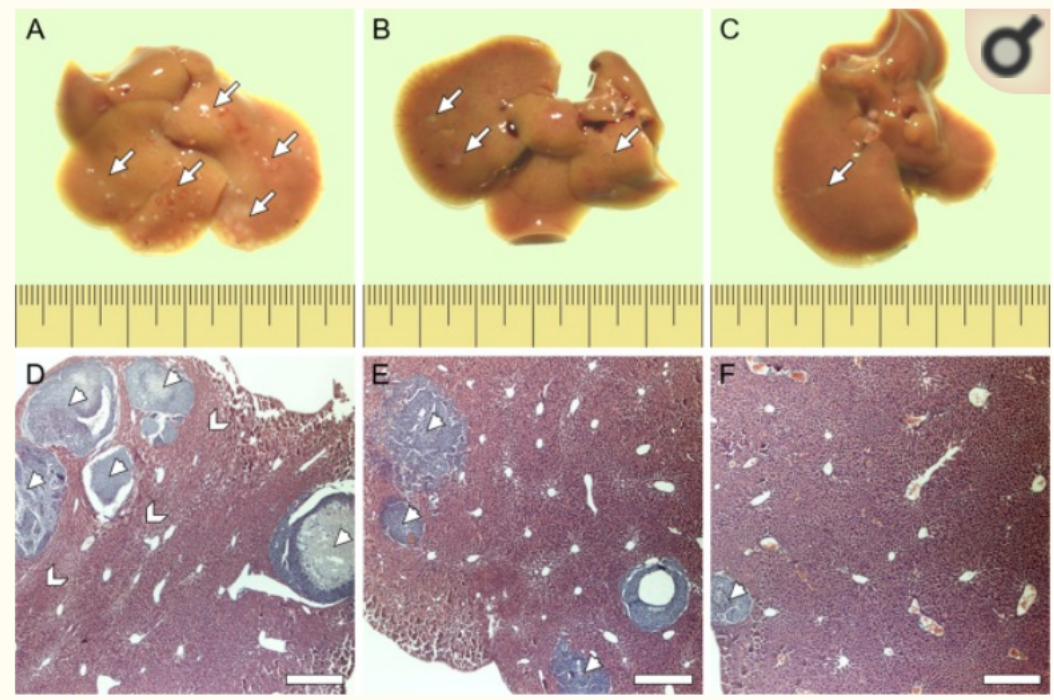

Figure 5.4 shows a visual concept of the livers that were obtained from the mice. Tissue samples obtained are listed as follows: (**A, D**) non pigmented cells, (**B, E**) moderately pigmented cells, and (**C, F**) heavily pigmented. [5]

PROLIFERATION OF MELANOMA CELLS

Lastly, the cells were also examined to see if there could be a relationship established between tumor size that were related to different proliferative abilities in the cells. **Figure 5.5** showed a comparison between the proliferation of the non-pigmented cells vs. pigmented cells. The graph indicated that the proliferation between the two showed no significant difference [5].

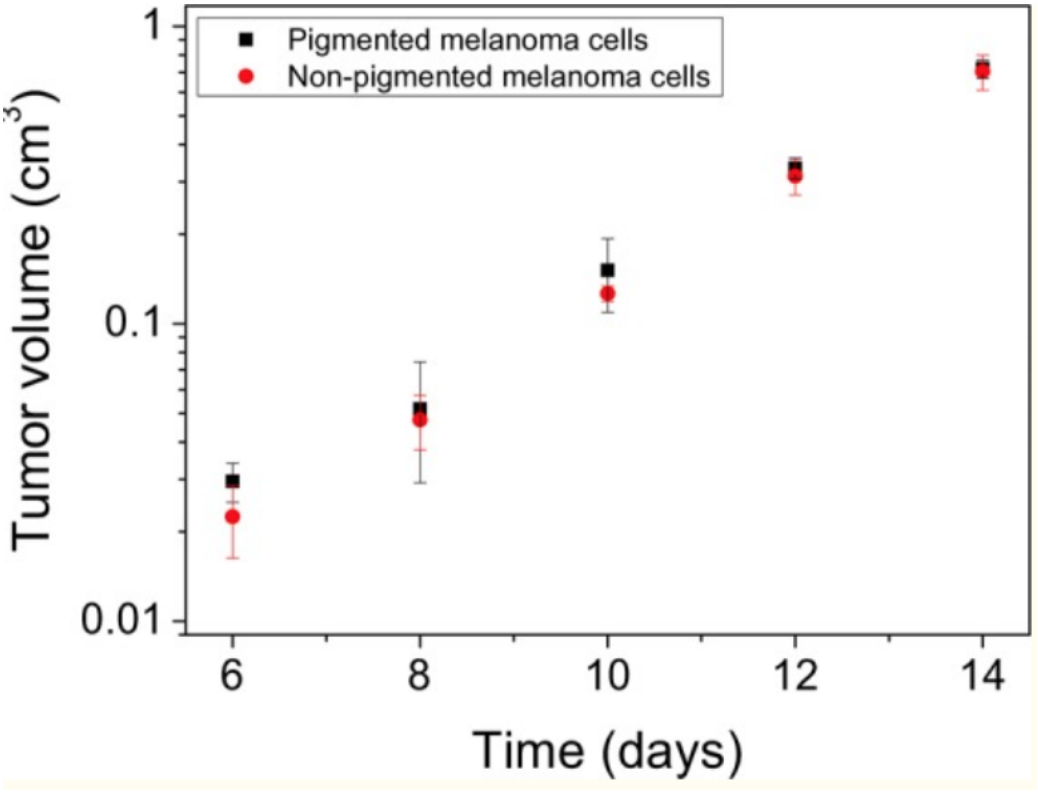

Figure 5.5 represents a growth curve of tumor progression between pigmented and non-pigmented cells. Tumor volumes were plotted over 14 days. [5]

CONCLUSION

Melanin plays a huge role in suppressing the effects of melanoma in humans. The mentioned studies have shown no correlation between the ethnicity of the patient and the number of melanocytes found in the body. Melanin was also shown to contain photoprotective properties that can be used to protect the skin from UV radiation, which could trigger a malignant transformation of the melanocytes in the body. Identifying melanoma's dependency on the cells' elastic properties helped expose the role melanin plays in the nanomechanical properties of the melanoma cells. This eventually led to the discovery that melanin concentration could cause the nature of melanoma to be less aggressive, due to the increase in stiffness of the cytoskeleton. Once that information was gathered, it opened the door to explore the benefits that came with the

production of melanin. The inhibition of melanoma metastasis by melanin was an immense breakthrough in science, as it deepened the understanding of melanin's role in preventing the incidence of melanoma. Continuing the research to unveil more secrets about melanin could further help doctors to properly diagnose patients and create a more efficient treatment for victims of melanoma skin cancer.

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Study of Ganglioside GM3 Association to Insulin Resistance Reveals Potential Therapeutic Drug via Inhibition of GM3S

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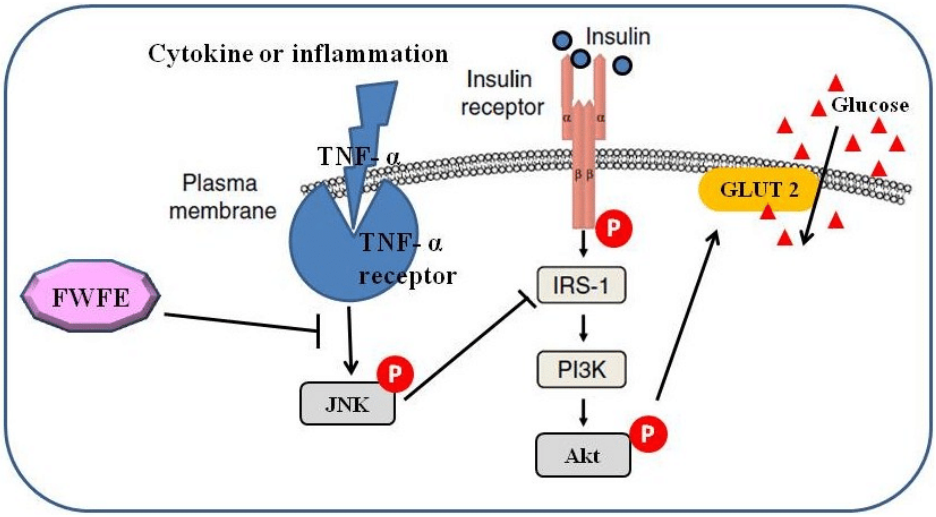
ABSTRACT

Ganglioside GM3 is a sialylated membrane-based glycosphingolipid that modulates insulin receptor signaling via direct association with the receptor. Insulin resistance induced by tumor necrosis factor-α (TNF-α) in 3T3L1 adipocytes is followed by the increased expression of GM3 through the elevation of GM3 synthase (GM3S) activity. This study demonstrates that TNF simultaneously produced insulin resistance via the uncoupling of insulin receptor activity toward insulin receptor substrate-1 (IRS-1), and subsequently suppressing insulin-sensitive glucose uptake. Moreover, exogenous GM3 incubated 3T3L1 adipocytes also suppressed the tyrosine phosphorylation of insulin receptor and subsequent IRS-1, and glucose uptake in response to insulin stimulation, demonstrating that GM3 alone can reproduce the effects of TNF on insulin signaling. Therefore, because high levels of GM3 and GM3S expression are observed in tissues of patients with diabetes, GM3S has been considered a therapeutic target for type II diabetes. However, there are no GM3S inhibitors discovered to date. This study also establishes a high-throughput scintillation proximity assay that can detect GM3S activity to further screen for GM3S inhibitors from an original chemical library and methods to detect the activity of GM3S and ST3Gal3 (another sialyltransferase) via the measurement of the enzyme products using RapidFire mass spectrometry. Thus, succeeding in identifying two different variants of GM3S-selective inhibitors characterized with mixed-mode inhibition. These compounds have the potential to be further developed into therapeutic drugs to treat or prevent type II diabetes.

INTRODUCTION

Insulin resistance can be defined as a disability of cells or tissues to respond to physiological levels of insulin and is a common condition of type II diabetes [3]. Many reports state that elevated levels of GM3 are seen in the sera of obese patients and the visceral fat of obese animal models [1]. Additionally, evidence points that tumor necrosis factor (TNFα) is a key mediator in insulin resistance linked to obesity via the down-regulation of the insulin-regulatable glucose transporter, GLUT4 [3].

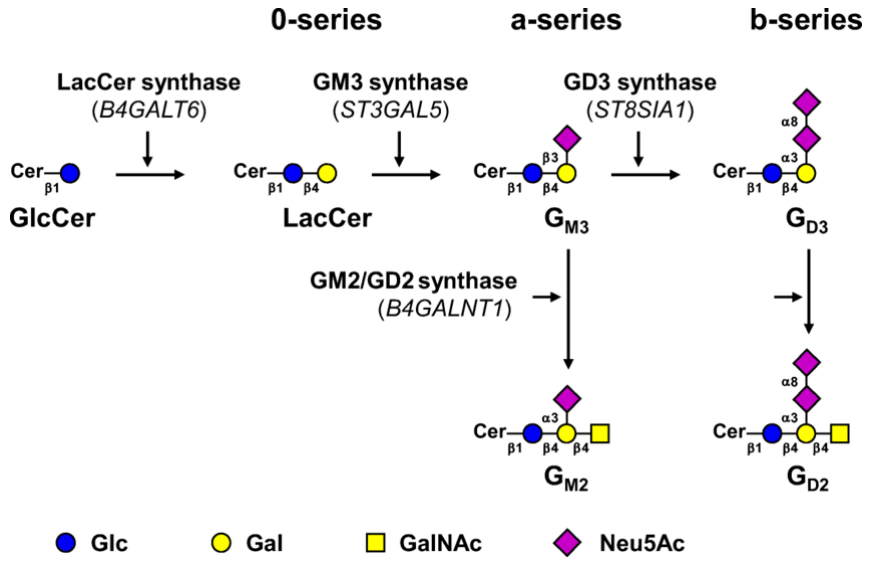


Chang, C. et al. (2019). Postulated mechanism of FWFE on modulating insulin signaling and inflammation pathway in TNF-α-induced insulin resistant FL83B cells [Image].

TNFα, on a cellular level, is a potent insulin signaling inhibitor that prevents the uptake of glucose [3]. It interferes with signaling through the insulin receptor and affects the substrates downstream, such as the insulin receptor substrate-1 (IRS-1), indicating that TNF ultimately produces a defect at or near the tyrosine kinase activity [3]. Therefore, it is important to determine whether TNF has an indirect or direct effect on the suppression of insulin function.

Gangliosides are sialic acid-containing glycosphingolipids that are transmembrane modulators [4]. They are involved in transducing cellular signaling through associations with

transmembrane receptor tyrosine kinases such as insulin receptors. Ganglioside GM3 is the most commonly distributed ganglioside in tissues [4]. It is the primary product in the biosynthetic pathway of the ganglio-series gangliosides, synthesized by the addition of cytidine-5′-monophosphate (CMP)-sialic acid to lactosylceramide (LacCer) by GM3 synthase (GM3S) [4].



Carvdarli, S. et al. (2019). Biosynthesis pathways for b-series GD2 and GD3 gangliosides [Image].

GM3S, also known as ST3Gal5, belongs to the ST3Gal group of the sialyltransferase family that functions to transfer sialic acid from CMP-sialic acid to substrates that contain galactose [4]. The GM3S gene expression is elevated in tissues such as visceral fat, skeletal muscle, and skin [4]. Therefore, mice with GM3S-knockout are expected to be born less susceptible to high-fat diet-induced insulin resistance [4].

These findings strongly suggest that GM3S can potentially be an effective therapeutic target for insulin resistance-associated diseases [4]. Although many studies have examined GM3 functions through direct approaches, such as exogenously adding GM3 to culture mediums, or indirect approaches, such as decreasing GM3 levels by using GlcCer synthase inhibitors to eliminate precursor glycosphingolipids, there are no GM3S inhibitors reported to date [4].

Moreover, inhibition of GM3S might represent a better outcome considering that GlcCer synthase-knockout results in the depletion of all types of glycosphingolipids and can lead to embryonic lethality [4]. Therefore, this paper examines the effects of the TNF enhancement and depletion of ganglioside GM3 on the control of insulin signaling, conducts a high-throughput screening (HTS) of a chemical library to identify novel GM3S inhibitors, and evaluates GM3S enzyme activity and its inhibitory activity to ultimately identify two chemotypes of selective GM3S inhibitors [3,4].

RESULTS

Effect of TNFα on Insulin-Receptor Signaling and Ganglioside Expression

TNFα binds to two receptors, the 55-kDa type 1 receptor and 75-kDa type 2 receptor [3]. Murine TNFα displays specificity to both receptors, whereas human TNFα is selectively recognizable by its association to the 55-kDa type 1 receptor [3]. Previous studies that used genetically obese mice showed that a knockout in the wild type TNF receptor and p75 receptor resulted in insulin resistance, but a knockout of the p55 receptor did not have the same results [3]. This demonstrated that TNF signaling through the p55 receptor is much more crucial in insulin resistance development than through the p75 receptor, which is why this experiment used human TNF to study the insulin signaling from the p55 receptor in murine 3T3-L1 adipocytes [3].

Since studies have shown that ganglioside metabolism is partly regulated by TNF signaling, the cell surface expression of GM3 (major ganglioside of 3T3-L1 adipocytes) was examined by flow cytometry using an anti-GM3 antibody (M2590) [3]. GM3 expression progressively increased after 0.1 nm TNF treatment; this increase was observed as early as three hours after treatment and high levels were maintained during long-term treatments as well

(**Figure 1A**) [3]. Similarly, it was observed that there was high cellular content of GM3 (**Figure 1B**) and GM3 synthase activity (**Figure. 1C**) due to TNF treatment [3].

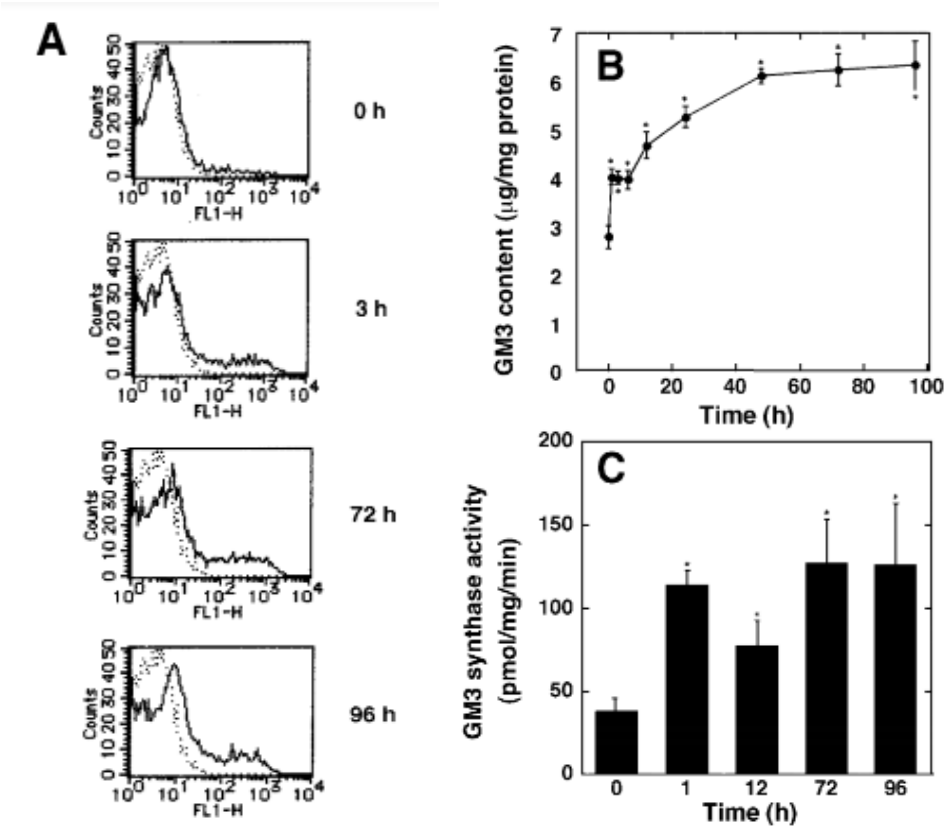


Figure 1. TNFα increases GM3 expression in 3T3-L1 adipocytes. (***Panel A***) Cell surface expression of GM3 was examined by flow cytometry using an anti-GM3 monoclonal antibody (M2590) after the adipocytes were treated with 0.1 nm TNF and maintained in the medium for varied time intervals (0-96h). (***Panel B***) GM3 content was analyzed by densitometry. This was done by preparing ganglioside fractions, separating them on TLC, and then finally, visualizing them using resorcinol staining. (***Panel C***) To measure GM3 synthase activity, cells were harvested and used as an enzyme source. [3]

Inhibition of Insulin Signaling by Exogenous GM3

The effect of exogenously added GM3 on tyrosine phosphorylation of IRS-1 in response to insulin stimulation is demonstrated in Figure 2 [3]. 3T3-L1 adipocytes that were incubated for twelve hours with 100 μM GM3 exhibited a significant decrease in tyrosine phosphorylation of IRS-1, which was not observed in GD1a minor ganglioside in 3T3-L1 cells (**Figure 2A**) [3].

Additionally, exogenous GM3 also displayed a marked decrease in the autophosphorylation of the insulin receptor and subsequent suppression of tyrosine phosphorylation of IRS-1 (**Figure 2B**) [3].

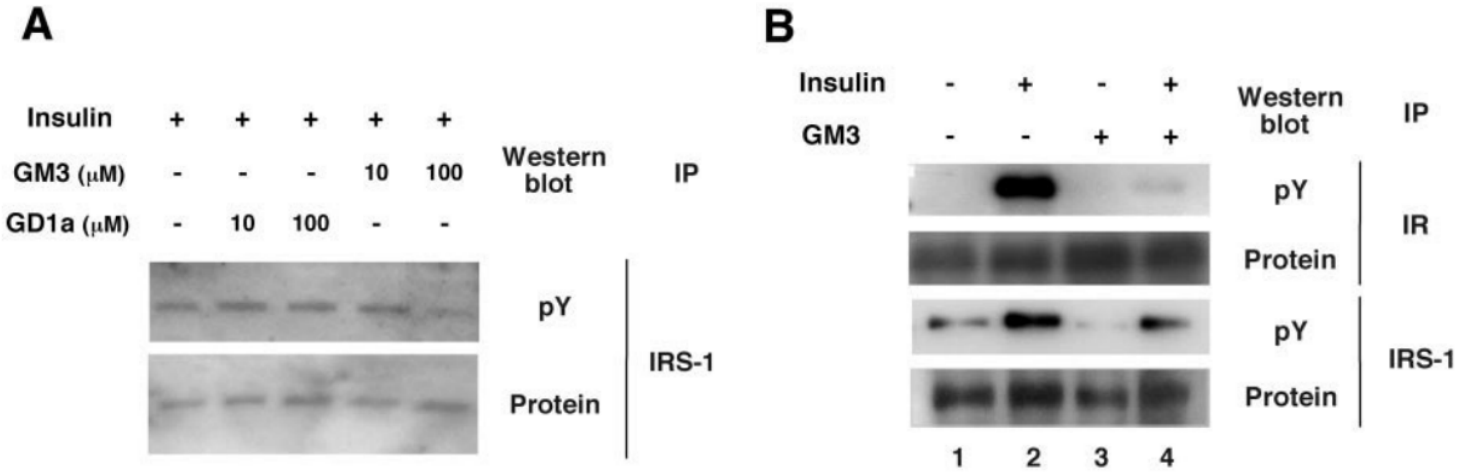


Figure 2. Insulin signaling is inhibited by exogenous GM3, not GD1a. (***Panel A***) 3T3-L1 adipocytes were incubated with/without 10 or 100 μM of GM3 or GD1a in 0.5% bovine serum albumin medium for 12 hours and then stimulated with 100 nM insulin for 3 min. Immunoprecipitation (IP) with antiserum to IRS-1 was performed on proteins in cell lysates, and then Western blotting with anti-phosphotyrosine (*p*Y) antibody and antiserum to IRS-1 was performed. (***Panel B***) 3T3-L1 cells were incubated without or with 100 μM GM3, stimulated with insulin, immunoprecipitated and Western blotted. [3]

GM3 Effects on Glucose Uptake

The exogenous addition of GM3, which resulted in the selective inhibition of insulin-dependent tyrosine phosphorylation of IRS-1, brings about a question as to whether GM3 can affect glucose uptake in 3T3-L1 adipocytes (**Figure 3**) [3]. Prolonged treatment of adipocytes with TNF is known to induce the suppression of glucose uptake, so cells that were cultured in 100 μM GM3 for 6 hours or 96 hours were compared for their glucose uptake activity [3]. Inhibition of glucose uptake was only observed in the 96 h treatment with 100 μM GM3; similar to the inhibitory effects observed when cells were incubated with 0.1 nm TNF for 96 h,

exogenous GM3 significantly suppressed glucose uptake in adipocytes [3]. This concludes that GM3 alone is able to reproduce a state of insulin resistance [3].

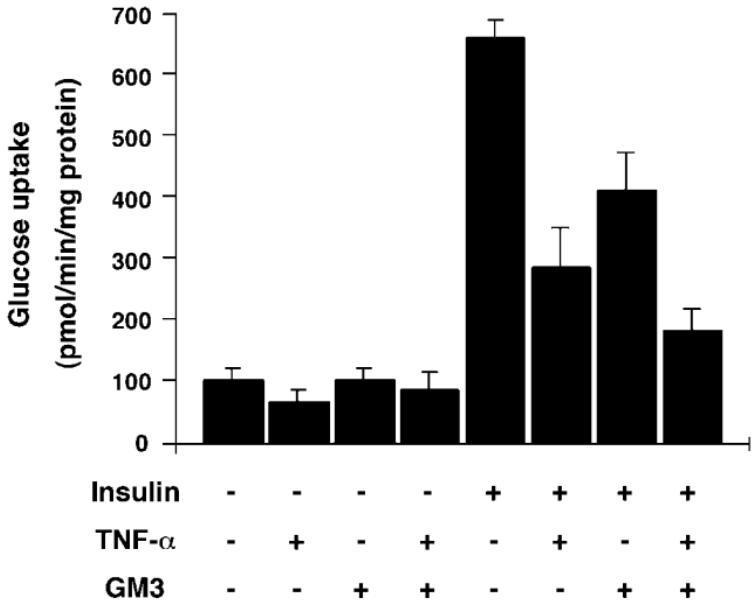


Figure 3. Inhibitory effects of exogenous GM3 on glucose uptake. 3T3-L1 adipocytes were cultured in a medium with or without 0.1 nM TNF and 100 μM GM3 for 96 hours. The cells were further cultured in a serum-free medium that contained 0.5% bovine serum albumin with the same doses of TNF and GM3 for 8h and then was stimulated with or without insulin for 20 minutes. [3]

TLC and SPA Developed to Detect GM3S Activity

GM3 levels were quantified via TLC to detect GM3S activity [4]. Sf21 microsomal fractions were used to express human GM3S as an enzyme source, and it was determined that GM3 levels and its rates of conversion from LacCer (precursor to most major glycosphingolipids) occurred in a concentration-dependent manner (**Figure 4A**) [4]. A high-throughput SPA was established based on these results, enabling the capture of the cell membranes that incorporated GM3 because GM3 is otherwise difficult to isolate from the membrane [4]. The SPA beads were used to measure the radiation signals derived from radioisotope-labeled CMP sialic acid that were converted to GM3 by GM3S in a

concentration-dependent and reaction time-dependent manner (**Figure 4 B&C**) [4]. Using 10 μM CMP-sialic acid and GM3S substrate, high signal-to-background ratios were obtained and confirmed the conversion rates determined via TLC (**Figure 4 D&E)** [4]. These results conclude that SPA and TLC can be used to quantify GM3S activity [4].

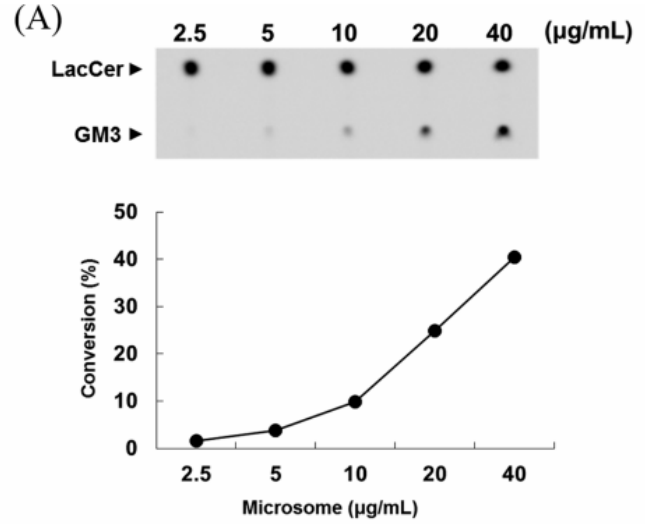


Figure 4 *A*. GM3 synthesis determined by TLC. GM3 synthesis occurs in an enzyme concentration-dependent manner. This was determined via TLC using human GM3S-overexpressed Sf21 microsomal fractions as the enzyme source with 10 μM nitrobenzoxadiazole (NBD)-labeled LacCer and 300 μM cytidine-5'-monophosphate (CMP)-sialic acid. [4]

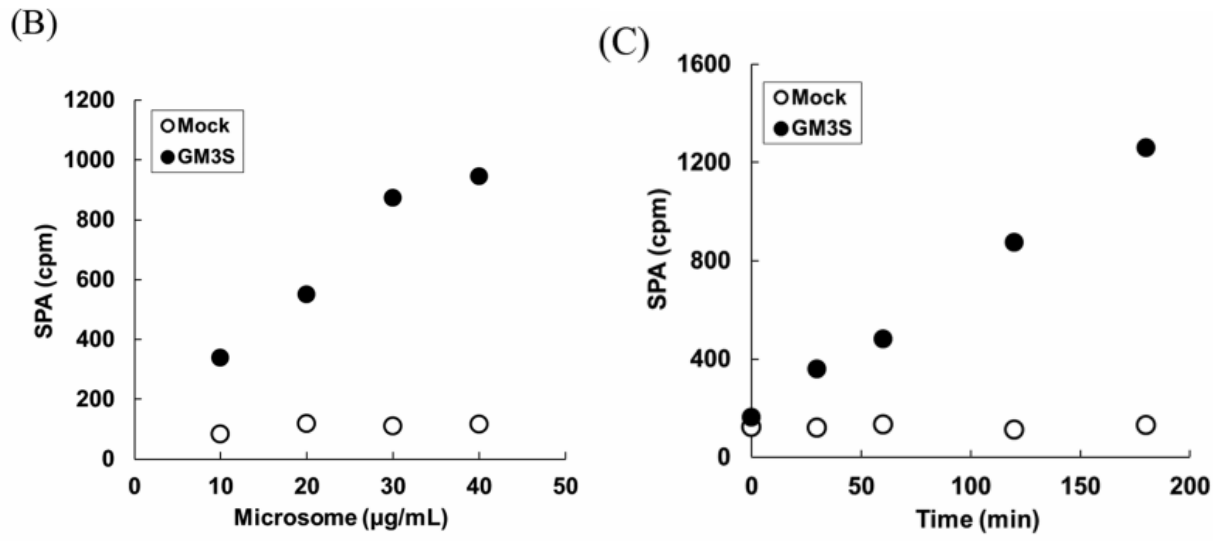


Figure 4 *B, C*. CMP-sialic acid conversion to GM3 by GM3S by SPA. SPA signals determine enzyme concentration-dependency, and reaction time-dependency increases with 10 μM LacCer and 10 μM CMP-sialic acid. [4]

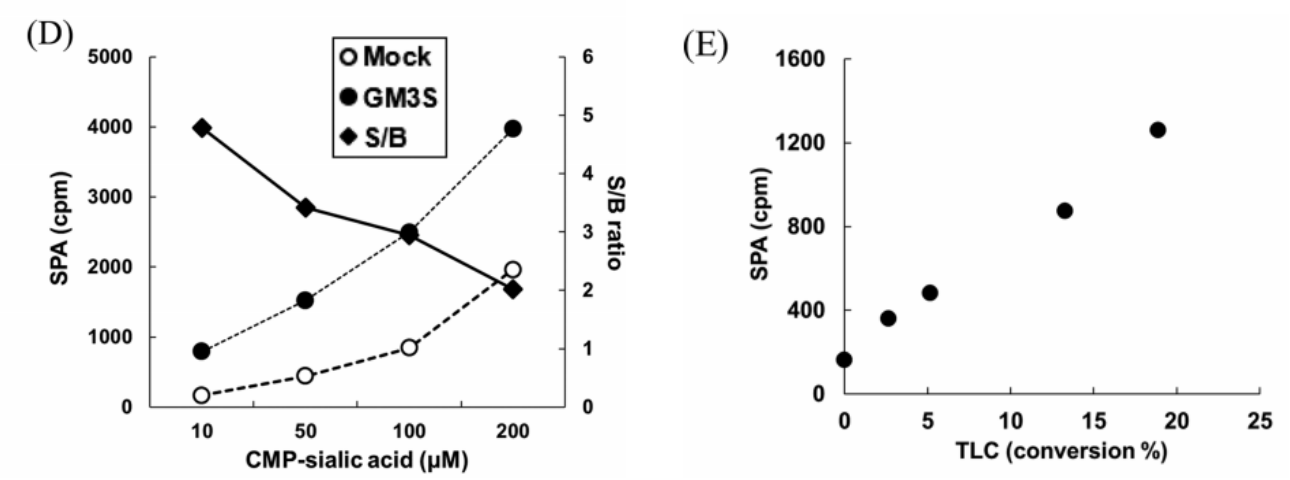


Figure 4 *D, E*. The effect of CMP-sialic acid addition on the signal-to-background ratios of SPA signals. This was performed under constant 10 μM CMP-sialic acid and *Panel E* confirms the correlation between SPA signals and substrate conversion rates via TLC. [4]

Identification and Characterization of GM3S-Selective Inhibitors

Based on the aforementioned results, a high-throughput screening (HTS) was conducted and presented in the flowchart (**Figure 5A**) [4] The second screening of the 3208 primary hit compounds was performed using RapidFire MS, and 144 compounds were selected with GM3S inhibitory activities of >24.5% [4]. Subsequently, 67 compounds with GM3S IC_{50} values <30 μM were chosen via dose-dependent analysis [4]. This indicates that only <30 μM of the inhibitory substance is needed to inhibit GM3S by 50% [4]. Thereafter, the selectivity assay with reaction buffer containing 2% DMSO obtained six compounds that had ST3Gal3 IC_{50} values >10-fold higher than those of GM3S, and this further aided in identifying two compounds, compound **1** and compound **2** [4]. Compound **1** and compound **2** had IC_{50} values of 13.0 μM and 15.6 μM against GM3S, respectively, while both had IC_{50} values >100 μM against ST3Gal3 (**Figure 5B**); this indicates that the compounds are GM3S-selective inhibitors [4].

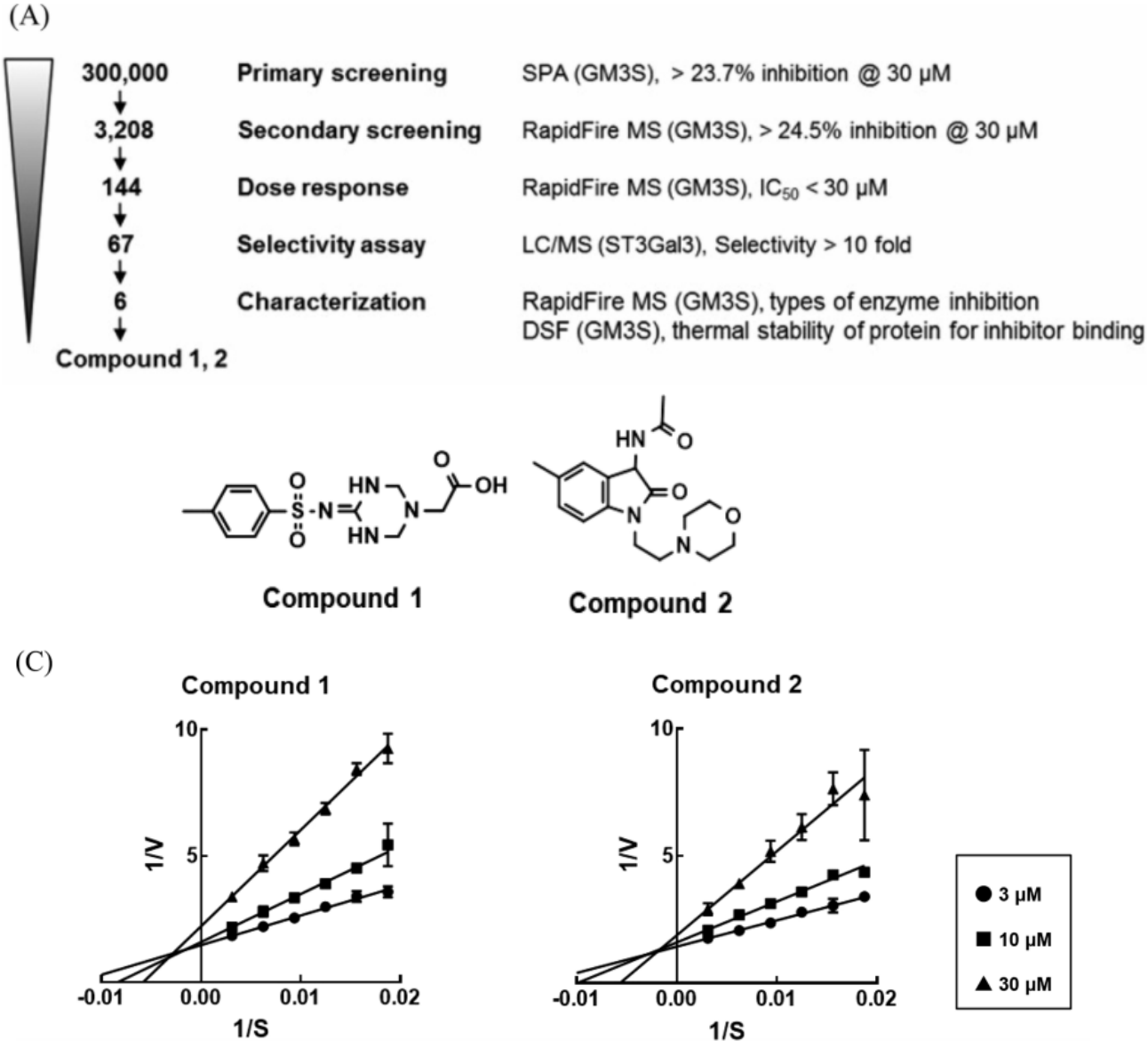


Figure 5. Identification and characterization of GM3S-Selective Inhibitors. (***Panel A)*** Shows the summary of the entire screening campaign and the chemical structure of compound **1** and compound **2**. (***Panel B***) Concentration-dependent inhibition of GM3S and ST3Gal3 activities by compound **1** and compound **2** were determined via RapidFire MS (GM3S) or Liquid Chromatography (ST3Gal3). (***Panel C***) Lineweaver-Burk plots of compound **1** (left) and compound **2** (right) under 53, 64, 80, 110, 160, or 320 μM CMP-sialic acid and 3, 10, or 30 μM of compound **1** and compound **2**. [4]

Additionally, a GM3S enzyme kinetic analysis using varying CMP-sialic acid concentrations was conducted to generate a Lineweaver−Burk plot (**Figure 5C**), which indicates that both inhibitor

compounds display a mixed-mode of inhibition [4]. This further suggests that compound **1** and compound **2** possibly interact with GM3S pockets, distinct from that which CMP-sialic acid binds to [4].

Finally, DSF assays were performed to demonstrate the direct relationship between the compounds and GM3S by monitoring the thermal stabilization of the protein upon ligand binding [4]. To go about this assay, purified GM3S that lacked 90 amino acids in the N-terminal transmembrane was prepared as a soluble protein, while a mannose-binding protein (MBP) was tagged to the N-terminus of the protein structure to increase its solubility (**Figure 6A**) [4]. The temperature curves and differential peaks of the protein interestingly revealed two different T_m values (the temperature at which the protein denatures); therefore, respective ligands (CMP for GM3S and maltose for MBP) were added to confirm whether the two peaks were derived from GM3S and MBP [4]. The addition of CMP increased the lower bound T_m value from 43 to 46 ℃ (**Figure 6B**), while the addition of maltose increased the higher bound T_m value from 61 to 64 ℃ (**Figure 6C**); this concludes that the lower T_m was associated with GM3S, whereas the higher value was associated with MBP [4]. Based on these results, the effects of compound **1** and compound **2** on the lower bound GM3S T_m value were investigated to reveal that these compounds decreased the T_m values in a concentration-dependent manner (**Figure 6E**) [4]. This suggests that both compounds induce conformational changes in the GM3S structure via direct binding, resulting in the possible destabilization of the protein [4]. The aforementioned data conclude that compounds **1** and **2** are authentic GM3S-selective inhibitors [4].

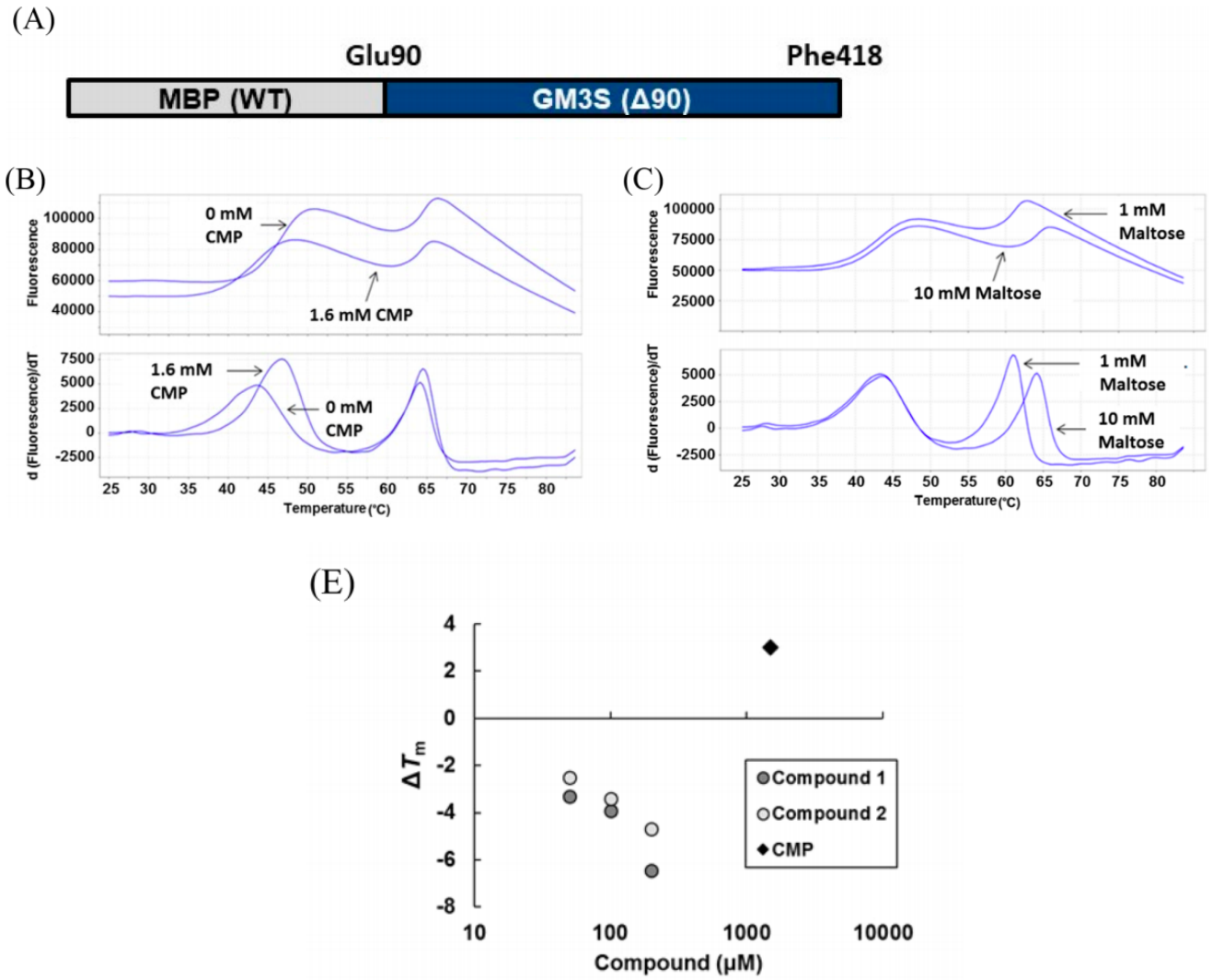


Figure 6. Using differential scanning fluorimetry (DSF) to evaluate GM3S binding capacity. (***Panel A***) Shows the protein structure of MBP-tagged GM3S used in the DSF assays. (***Panel B, C***) Temperature denaturation curves of MBP-GM3S in the presence or absence of 1.6 mM CMP and 10 mM maltose, respectively. (***Panel E***) Change in ΔT_m of MBP-GM3S after compound 1 (50, 100, and 200 μM), compound 2 (50, 100, and 200 μM), or CMP (1500 μM) was added. [4]

DISCUSSION/CONCLUSION

GM3 is the primary product in the ganglioside biosynthetic pathway [4]. High levels of GM3 have been correlated to insulin resistance and associated diseases, as previous studies have observed that a higher expression of GM3 had an inhibitory effect on insulin receptors [3]. This further indicates a possible cause of insulin resistance in type II diabetes [4]. Moreover, the link

between insulin resistance and the onset of type II diabetes is consistent when TNF-α is involved as the central mediator [3]. High doses of TNF in adipocytes result in the suppression of most lipogenic enzymes, stimulates dedifferentiation, and reduces the expression of insulin receptor signal transduction components like IRS-1 genes [3]. The study demonstrates that the state of insulin resistance in adipocytes under 0.1 nm TNF treatment resulted in the progressive increase of cell surface GM3, cellular GM3 content, and GM3S activity, indicating that TNF is involved in the transcriptional upregulation of GM3 synthesis in adipocytes [3]. To further elucidate that high levels of GM3 in adipocytes under TNF treatment correlate to insulin resistance, the effect of the exogenous addition of GM3 on tyrosine phosphorylation of insulin receptor and IRS-1 was examined [3]. Autophosphorylation of the insulin receptor and subsequent tyrosine phosphorylation of IRS-1 was significantly reduced only when adipocytes were incubated with 100 μM of GM3, and not GD1a (a minor ganglioside in the cell line) [3]. Therefore, if a state of insulin resistance in adipocytes can be produced through the inhibition of insulin receptor to IRS-1 signaling by the exogenous addition of GM3, it can be assumed that the downstream uptake of glucose is also affected [3]. This was proven when prolonged treatment of GM3 resulted in the suppression of insulin-sensitive glucose uptake, showcasing that GM3 alone is able to reproduce a state of insulin resistance [3]. This discovery is vital, and regulation of GM3 levels may be the key to a potential treatment of insulin resistance and its associated diseases.

Since GM3 is directly synthesized by GM3S to serve as a precursor to further complex gangliosides, the inhibition of GMS3 may mechanistically lead to a potential treatment of type II diabetes [4]. This study also established a novel method for screening GM3S inhibitors [4]. The SPA method was selected because GM3 is otherwise difficult to isolate from the membrane; the usage of WGA-conjugated SPA beads allows for easy and homogenous capture of them, further

facilitating HTS [4]. The net inhibitory effects of the hit compounds were confirmed by directly measuring GM3S and ST3Gal3 products through the use of MS, consequently resulting in the identification of two different variants of GM3S inhibitors (compound **1** and compound **2**) [4]. Both compounds had selectivity against ST3Gal3 that exceeded 10-fold, while both had less than 16 μM against GM3S; this indicated that both compounds are GM3S-selective inhibitors [4]. Furthermore, both compounds exhibited a mixed-mode of inhibition, suggesting that they may bind to allosteric pockets of GM3S [4].

Compound screening through the application of original methods was used to discover GM3S-selective inhibitors, which have the potential to be developed into drugs [4]. Future experiments would include the development of these inhibitors to increase their potency and selectivity, and improve their physicochemical properties, such as lipophilicity, by studying the structure−activity relationships prior to cell-based studies and/or animal testing [4].

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Survival and Selective Reversibility of Latent HIV Infection in Microglial Cells

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ABSTRACT

Human Immunodeficiency Virus (HIV) is a virus that attacks the immune system by destroying immune cells. However, recent studies have shown that HIV can exist in a latent form that helps them survive apoptosis. It has been discovered that microglial cells are the main reservoirs of HIV in the brain. The latent cells are capable of becoming activated and causing HIV infection. To obtain a functional cure for HIV, it is essential to target all the HIV cells, especially the latent cells. However, targeting microglial cell reservoirs is a major obstacle. Current therapies pose limitations because of the inability to penetrate the blood-brain barrier (BBB), the lack of clinical tests, and the obstacle to identifying latent reservoirs. Furthermore, the mechanism through which HIV helps cells survive apoptosis is not fully understood. However, recent research has helped obtain a better understanding regarding the factors that cause HIV latency and help the latently infected cells survive apoptosis, potentially leading to the discovery of a functional cure. This paper aims to analyze the research on the discovery that microglial cells are reservoirs, the persistence of HIV latency in these cells that causes them to survive apoptosis, and the use of Bim as a potential biomarker. The paper will also discuss using a humanized mouse model to identify compounds that reverse HIV latency and the effect of neurons on the survival of HIV infection in microglial cells. Lastly, it will mention the limitations of current therapies when targeting HIV-infected microglial cells.

INTRODUCTION

Human Immunodeficiency Virus (HIV) targets and destroys immune cells to damage the immune system. It mainly spreads through unprotected sex with an HIV-positive person [7]. Even though there is no functional cure for HIV yet, there are many medicines and strategies used to help fight and reduce the risk of spreading the HIV infection [7]. However, these medicines and strategies pose a challenge when targeting infected cells. It has widely been studied that CD4+ T cells are the main reservoirs of HIV. However, many other cell reservoirs of HIV are not yet well researched, such as microglial cells and monocyte-macrophage lineage cells [4]. It is considered that HIV first targets the monocyte-macrophage lineage cells, further rapidly spreading the virus by transmitting to CD4+ T cells [6]. The macrophages harbor the virus in cell membrane invaginations and protect the virions from antiretroviral therapy (ART) and circulating neutralizing antibodies, allowing them to survive for extended periods [10]. However, the mechanisms through which HIV-infected macrophages can survive for extended periods and transmit the virus are still not fully known [2].

Even though most of the HIV-infected cells undergo apoptosis, some can become latent viral reservoirs, making them resistant to apoptosis [11]. The criteria for being latent viral reservoirs include extended survival, silencing of viral replication, and reactivating replication under specific treatments [3]. One of these reservoirs is the microglial cells. Glial cells are the primary most abundant cells found in the central nervous system, functioning as the brain's immune cells [9]. They function to support synaptic contacts, help maintain the signaling of neurons, and aid in removing cellular debris [9]. HIV reservoirs that are resistant to antiretroviral therapy (ART) are a significant obstacle to curing HIV [2]. Furthermore, these reservoirs' latency makes it more challenging to identify and target the cells, especially the microglial cells. Therefore, strategies

that target all latent HIV reservoirs by either removing, disabling, or suppressing the reservoirs are required to cure HIV [5].

The studies about microglial cells and HIV are relatively new. This review will first discuss the experiments performed to establish microglial cells as HIV reservoirs, address how they can survive apoptosis, and examine how to identify HIV reservoirs *in vivo* using Bim as a potential biomarker. This will be followed by a study about the humanized mouse model used to identify compounds that selectively reverse HIV latency, and the effect of neurons on the survival of HIV infection in microglial cells. Lastly, it will discuss the three current therapies used to control HIV and the limitations of each when targeting the microglial cells.

LATENTLY INFECTED MICROGLIAL CELL RESERVOIRS CAN SURVIVE APOPTOSIS

Previously, researchers have mainly correlated HIV reservoirs with CD4+ T cells. However, recent studies have shown that microglial cells can also fit the criteria of HIV reservoirs. Prior to analyzing the mechanism of apoptosis survival in HIV-infected microglial cells, it was first essential to examine if microglial cells can become viral reservoirs [2]. Uninfected and HIV-infected cells were quantified for survival for up to 120 days using isolated human fetal microglial that was infected with HIV and stained with DAPI (to observe nuclei), TUNEL (to observe apoptosis), and HIV-p24 (to detect HIV infection) [2]. The uninfected cultures had higher survival rates initially; however, after 21 days post-infection, the cells experienced significant apoptosis [2]. In contrast, the HIV-infected cultures had fast initial apoptosis up until 21 days [2]. After 21 days post-infection, HIV-infected cultures' survival rate was significantly higher and stable than the uninfected cultures [2]. Since the HIV-infected cultures included both uninfected cells and HIV-infected cells, it was important to determine which type of cells were surviving HIV infection [2]. Therefore, the HIV-infected cultures were stained with DAPI and HIV-p24 [2].

The majority of the surviving microglial cells were HIV-p24 positive, indicating that the HIV infection was responsible for the extended survival of HIV-infected microglial cells [2].

Once it was determined that the HIV infection helps a small population of microglial cells survive apoptosis, HIV replication was examined within the infected microglial cells [2]. The levels of HIV-p24 were measured in both the uninfected cultures and the HIV-infected cultures. The uninfected cultures showed no presence of HIV-p24, whereas the HIV-infected cultures showed the highest measure of HIV-p24 between 28 to 36 days post-infection [2]. However, the level of HIV-p24 decreased to undetectable levels after 120 days post-infection, indicating that the HIV replication in microglial cells becomes silent [2]. To further show that the surviving HIV-infected microglial cells were latently infected, the HIV replication was reactivated after 120-150 days post-infection [2]. The microglial cells were treated with several factors such as SAHA, PHA, Meth, LPS, and a combination of TNF-α and IFN-γ [2]. An increase in the production of HIV-p24 was observed with the use of these treatments, indicating that the HIV replication was reactivated [2]. Therefore, since HIV-infected microglial cells survive apoptosis, silence HIV replication, and can be reactivated under specific treatments, they fit the criteria of HIV reservoirs [2]. Since the following experiment was performed using human macrophages, due to the limited number of isolated microglial cells, it was important to show that macrophage reservoirs are similar to microglial cell reservoirs [2]. Therefore, the macrophages were also quantified for survival and stained with DAPI, TUNEL, and HIV-p24 [2]. Like HIV-infected microglial cells, HIV-infected macrophages were also able to survive apoptosis and become latently infected, indicating that macrophages' findings can be assumed for microglial cells as well [2].

After confirming that microglial cells fit the criteria for HIV reservoirs, it was essential to determine how HIV promotes the survival of these viral reservoirs. For apoptosis to occur, it is vital to form a transition pore in the mitochondrial membrane and release mitochondrial factors into the cytoplasm [2]. The expression of proteins involved in mitochondrial outer membrane (MOM) pore integrity was analyzed and only Bim was seen to be upregulated in latently HIV-infected macrophages when compared to Bim levels in uninfected cultures [2]. The lack of expression of any other protein indicated that a transition pore formation is not possible. However, since Bim is a pro-apoptotic protein being upregulated in cells that survive apoptosis, it can be considered that HIV might block its apoptotic function [2]. Furthermore, the protein expression levels of factors that trigger apoptosis, such as cytochrome C (CytC), caspase-3, and apoptosis-inducing factor (AIF), were also examined in response to HIV infection [2]. No change in expression or activation of caspase-3 and in the molecular weights of apoptotic proteins were detected, further confirming apoptosis is blocked [2]. Lastly, it was important to determine if the mitochondrial factors AIF and CytC are secreted into the cytoplasm using cell fractionation in both uninfected and HIV-infected cells [2]. Both AIF and CytC were retained inside the mitochondria in latently infected cells; therefore, blocking apoptosis [2]. The similarities between macrophage reservoirs and microglial cell reservoirs can help assume that HIV also blocks the formation of the transition pore and the secretion of the mitochondrial factors into the cytoplasm of the latently infected microglial cells. Therefore, the latently infected microglial cell reservoirs survive apoptosis. Interestingly, Bim was present in mitochondria during the cell fractionation and was highly upregulated *in vitro* [2].

BIM AS A POTENTIAL BIOMARKER

Bim is a pro-apoptotic protein that is recruited into the mitochondrial membrane to trigger apoptosis during apoptosis activation [2]. However, it was observed that Bim was upregulated and recruited into the mitochondria in latent HIV-infected macrophages where apoptosis is blocked *in vitro* [2]. To determine if these findings of Bim *in vitro* would be consistent *in vivo*, immunostaining for Bim, VDAC (a mitochondrial protein), HIV-p24, and DAPI was performed [2]. Human tissues from lymph nodes and brains were obtained from uninfected and HIV-infected individuals on effective ART at the time of death [2]. It was noted that the HIV-infected tissues had undetectable viral replication, indicating they were latently infected [2]. No staining for HIV-p24 and diffuse Bim and VDAC staining was observed in the uninfected tissues for both lymph nodes and brains [2]. In comparison, the HIV-infected tissues for both lymph nodes and brains showed increased Bim expression in perfect colocalization with VDAC [2]. Therefore, the data of Bim *in vitro* was consistent with the data of Bim *in vivo* [2]. Since the upregulation of Bim and recruitment to the mitochondria in latently infected microglial cells *in vitro* and *in vivo* does not result in apoptosis, Bim can be a potential biomarker to identify HIV reservoirs *in vivo* [2]. Targeting latently infected microglial cells has always posed an obstacle due to viral replication being silent. Therefore, the discovery of Bim as a potential biomarker *in vivo* marks an important milestone towards identifying microglial cell reservoirs and targeting them to cure HIV.

BIX01294, UNC0638, AND PHENELZINE CAN SELECTIVELY REVERSE HIV LATENCY IN MICROGLIAL CELLS

The latency of HIV infection is a crucial step towards the survival of apoptosis. However, is it possible to reverse HIV latency in microglial cells? Two methods were used to condition

adult mice and create humanized NSG (NOD scid gamma) mice: total body irradiation and busulfan (a chemotherapeutic agent), both of which ablated the endogenous cells of the mouse [8]. The mice were then transplanted with human CD34+ HSC that were obtained from a single source to avoid cell variation [8]. The mice were euthanized and perfused with PBS 16 weeks post-transplantation to avoid contaminating brain cells from the blood cells [8]. Using Percoll gradient, the glial fraction was isolated from brain tissue and immunostained for hCD45 (human-specific marker), and CD11b and P2RY12 (microglial markers) [8]. Compared to the irritated-conditioned mice, the busulfan-conditioned mice showed an increase in the frequency of human cells in both the glial fraction and the total microglial population, indicating the transplantation of human microglial cells in the mice was successful [8]. Five humanized mice were then infected with JRCSP-HA (an R5-tropic strain of HIV) to examine if the human microglial cells in the mice could be infected with HIV *in vivo* [8]. The JRCSP-HA was chosen due to its ability to identify infected cells by expressing hemagglutinin (HA) epitope-tagged protein [8]. The levels of virus in the blood were monitored for 12 weeks before the mice were necropsied to isolate the microglial cells and stain them with markers hCD45, CD11b, and HA [8]. Roughly 2-15% of the cells expressing markers hCD45+ and CD11b+ were positive for HA, indicating that the microglial cells in the brain of humanized mice were infected with HIV [8].

The mechanisms that promote HIV latency in CD4+ T cells are well understood; however, the mechanisms that promote HIV latency in microglial cells are not [8]. The CHME-5/HIV cells, "a rat microglial cell line that contains a single integrated copy of a defective reporter HIV genome [where] Nef has been replaced by GFP reporter," were subjected to an shRNA screen to identify genes involved in maintaining the HIV latency [8]. The three specific shRNA genes in the CoREST complex, a chromatin-modifying corepressor complex that

regulates neuronal gene expression and neuronal stem cell fate, that ranked as high hits in the shRNA screen were HDAC2, CTBP1, and CTBP2 [8]. The HC69 microglial cells were treated with pharmacological inhibitors to target specific members of the CoREST complex; the inhibitor BIX01294 targeted G9a, inhibitor UNC0638 targeted GLP/G9a, and inhibitor Phenelzine targeted LSD1 [8]. The GFP expression (active HIV expression) of HC69 cells was measured in untreated and treated cultures to examine the inhibitor's effect on the latency of HIV [8]. The three inhibitors showed an increased GFP expression in cells compared to the untreated cells, indicating they are latency-reversing agents in HC69 cells [8]. The inhibitors BIX01294 and UNC0638 were also tested on T cells and monocytes; however, GFP expression barely increased compared to the control, confirming that these latency-reversing agents are specific for microglial cells [8]. Phenelzine is a known inhibitor of monoamine oxidase (MAO); however, it is also an inhibitor of LSD1, a CoREST complex member [8]. To determine whether reactivation of HIV with Phenelzine is due to its anti-LSD1 activity or anti-MAO activity, the cells were treated with specific MAO inhibitor (M-30) and LSD1 inhibitors (RN-1, GSK-LSD1, and SP-2509) [8]. The specific LSD1 inhibitors had minimal effect on the GFP expression, while the specific MAO inhibitor significantly increased GFP expression [8]. This confirmed that the anti-MAO activity is responsible for the Phenelzine-mediated reactivation of latent HIV in microglial cells [8]. Phenelzine was further used in the NSG humanized mice, and it was observed that Phenelzine resulted in a roughly two-fold increase in the HIV release compared to untreated cells [8]. Therefore, with the use of drugs BIX01294, UNC0638, and Phenelzine, it is possible to reverse HIV latency in microglial cells *in vitro* and *in vivo*, which can either initiate apoptosis in the cells or make it easier to target the viral reservoirs with current therapies by actively expressing HIV.

The Correlation Between Neurons And Hiv Expression In Microglial Cells

It has been widely suggested that HIV latency in the brain could also be related to the interactions between microglial cells and neurons [1]. A co-culture of LUHMES-derived neurons and HIV-infected microglial cells was established to determine how neurons impact HIV expression in microglial cells [1]. LUHMES cells, a human mesencephalic cell line, contain a vector that expresses red fluorescent protein (RFP), which can help visualize the neurons [1]. The LUHMES cells were expanded in neuronal growth medium (NGM) for four days and then placed in a modified neuronal differentiation medium (mNDM) for one day to hinder "the expansion of neuronal precursors and induce differentiation into dopaminergic neurons" [1]. The immortalized human HIV-infected microglial cells were then plated on top of the neurons in three neurons-to-microglial cells ratios: 10:6, 25:6, and 50:6 [1]. The HC69 cells contained both GFP^+ (actively infected) and GFP^- (latently infected) cells [1]. The impact of neurons on HIV expression in cultures without neurons (control), 10:6 ratio, 25:6 ratio, and 50:6 ratio was examined after 24 hours using flow cytometry and fluorescence microscopy [1]. HIV expression seemed to decrease as the ratio of neurons-to-microglial cells increased, indicating neurons silence HIV in a density-dependent manner [1]. Furthermore, to examine the impact of neurons on viral replication, GFP^- HC69 cells were co-cultured in the absence or presence of LUHMES-derived neurons for 72 hours [1]. A 13% increase in GFP expression was observed in the absence of neurons, whereas the presence of neurons kept the GFP expression at basal levels; this was indicative that neurons prevent HIV emergence from latency [1]. However, it was important to examine whether damaged neurons behave in the same way.

To examine the effect of neuronal damage on HIV expression, HC69 were co-cultured for 24 hours with healthy neurons or damaged neurons with a treatment of 0.05% trypsin, and

analyzed using flow cytometry [1]. The HIV expression in microglial cells with healthy neurons significantly decreased compared to HIV expression in microglial cells alone [1]. In contrast, HIV expression in microglial cells with damaged neurons increased compared to control [1]. Therefore, damaged neurons increase HIV expression and have the opposite effect on the HIV expression in microglial cells compared to healthy neurons [1]. Lastly, the impact of HIV on neurons for an extended period was examined by co-culturing LUHMES-derived neurons in the absence or presence of C20 microglial cells (uninfected parental cell line) or HC20 microglial cells (infected cells) for 96 hours [1]. The neurons were stained with either anti-beta-TUJ antibody (used to detect axons) or anti-MAP2 antibody (used to detect dendrites) and DAPI [1]. The HC20 cells had a slight decrease in the number of beta-TUJ positive cells than the control, indicating the axons were preserved [1]. However, the HC20 cells had a significant decrease in the number of MAP2 positive cells than the control, indicating that long-time exposure to HIV resulted in extensive dendritic pruning [1]. Healthy neurons silence HIV and prevent reactivation of viral replication; however, damaged neurons have the complete opposite effect. Furthermore, overexposure to HIV-infected microglial cells results in neuronal damage, which can then induce HIV expression in the microglial cells and lead to further problems like HIV-associated neurocognitive disorders (HAND).

Challenges Of Using Current Therapies To Target Microglial Cells

To ensure a functional cure of HIV, targeting all the reservoirs, including microglial cells, is essential. The production of the virus in microglial cells is responsible for HIV resistance to apoptosis [11]. However, targeting these cells is challenging due to the limitations of current therapies. Current therapies have limited efficiency due to “the intrinsic nature of the strategy, the nature of the cells targeted, or the attributes of the virus to escape antiviral responses” [11].

These limitations make it harder for all the reservoirs to be targeted, especially microglial cells. The blood-brain barrier (BBB) limits the access of drugs to reach the target. Even though new drugs have been produced to penetrate the BBB, serious side effects have been discovered [11].

Three primary therapies that have been tested include the Shock and Kill strategy, the Block and Lock strategy, and the gene therapy [11]. The Shock and Kill strategy focuses on reactivating the latent virus, while the Block and Lock Strategy focuses on achieving long-term control over HIV infection by inhibiting the gene expression [11]. Gene therapy concentrates on building HIV-resistant cells using nuclease-mediated gene-editing tools [11]. However, all of these therapies pose a limitation when targeting microglial cells. The Shock and Kill strategy reactivate microglial cells leading to neuroinflammation due to the release of neurotoxic pro-inflammatory factors, which can only be overwhelmed if the drug prevents neuroinflammation efficacy while reactivating the reservoirs [11]. The Block and Lock strategy is considered as an alternative to the Shock and Kill strategy, resulting in insufficient research on drugs that can penetrate the BBB [11]. A hypothesized strategy to overcome the limitations of both the Shock and Kill strategy and the Block and Lock strategy discusses the use of the Shock and Kill strategy to reactivate the reservoirs to decrease the number of latent reservoirs [11]. This is followed by the Block and Lock strategy that targets the persisting reservoirs to repress transcription and prompt deep latency [11]. This combination might be the key to reducing all HIV reservoirs, including microglial cells, to where no further treatment is necessary for a cure [11]. The gene therapy is relatively new; it has mainly been tested *in vitro*. However, this therapy's limitation is also the drug penetrance in the CNS and the ability of HIV-1 to evolve resistant strains of CRISPR/Cas9, initially responsible for suppression [11]. To target the reservoirs in microglial cells, ongoing research is on the lookout for new specific biomarkers that could lead

to the development of strategies targeting brain reservoirs. Despite this, testing these biomarkers *in vivo* will be extremely difficult and will pose a challenge of its own [11]. However, due to the production of HIV in the CNS, it is essential to target the reservoirs in the brain along with the other reservoirs to obtain a functional cure for HIV.

CONCLUSION

Microglial cells are viral reservoirs of HIV that can survive apoptosis by becoming latently infected, making it harder to identify and target these cells. HIV blocks the process of apoptosis in the surviving latently infected cells as no secretion of mitochondrial factors into the cytoplasm, and no formation of transition pore was observed. A unique feature of the surviving latently infected microglial cells was the significant upregulation and recruitment of Bim into the mitochondria, suggesting the potential use of Bim as a biomarker to identify HIV reservoirs *in vivo*. Since the latency of HIV infection in microglial cells aids them to survive apoptosis, the humanized mouse model helped identify compounds BIX01294, UNC0638, and Phenelzine to reverse HIV latency selectively. Reactivation of latent HIV reservoirs by specific drugs is being considered as an alternative to ART (for the reservoirs that are resistant to ART) to potentially reduce the latent reservoirs and target these reservoirs using current therapies/strategies. Healthy neurons were seen to silence HIV expression and prevent the re-emergence of HIV from latency, which can help protect the latent reservoirs from being targeted. However, overexposure to HIV-infected microglial cells resulted in neuronal damage, leading to an increase in HIV expression and potential HAND. Therefore, it is crucial to identify and target the microglial cell reservoirs within a specific time period so that over-exposure to HIV infection does not lead to other disorders, such as HAND, and also to cure HIV fully. These studies' findings have opened doors for future research to obtain a functional cure for HIV by targeting all the reservoirs,

especially the microglial cells. Future research could use microglial cells to perform experiments that confirm whether the mitochondrial factors are retained in the mitochondria or secreted to the cytoplasm. Moreover, the therapeutic effect of the reversing agents BIX01294, UNC0638, and Phenelzine can be evaluated by reactivating the viral replication and using current therapies/strategies to target the reservoirs to observe the effectiveness of treatment with and without latency. Additionally, reactivating the viral replication could also be used to examine the effect of Bim regulation in active cells compared to Bim regulation in latent cells in hopes of better understanding why Bim is upregulated without being functional.

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Depression and Suicidality in Adolescence

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ABSTRACT

Depression and suicidality among adolescents have been on the rise for the past 30 years. Varying attributing factors that have led to the increase in suicidality among adolescents and comorbid attributes have been linked to depression. Adolescents have been found to suffer from depression more than other age groups and have been found to have the highest suicide rate among other age groups. Contributing factors for suicidality include depression, bullying, socioeconomic status, education, and family support. Many different approaches have been carried out when treating depression and suicidality among adolescents such as group counseling and Interpersonal Psychotherapy-Adolescents (IPT-A). Primary care doctors have also reported on numerous occasions and across several countries that they have not been properly trained to handle mental health among their patients and would like to be provided with resources for optimal care. Depression and suicidality are two of the most leading problems that adolescents have been facing for the past 30 years, and the statistics presented in this paper show that these problems continue to rise.

WHAT IS DEPRESSION?

Depression is a mental illness that affects a significant portion of the world population; it transcends cultural boundaries, socioeconomic status, skin color, and even religion. It affects a disproportionate number of adolescents, and has consequently made suicide the third-leading cause of death in the United States [20,7]. In this literature review, the following topics will be discussed in greater detail: treatment for depression and suicidality, statistics of depression and suicidality, and statistics of adolescent boys, girls, and LGBTQ youth who suffer from depression and suicidality. This review will also discuss the definitions and symptoms of depression, risk factors for suicidality, major contributing factors to depression and suicidality, and comorbidity of depression with other mental illnesses.

Depression, according to the DSM-V, is characterized by the presence of five or more specific symptoms. The continuance of the following symptoms for a 2-week period (nearly every day and all day) is required for diagnosis: (1) depressed mood, (2) feelings of sadness, emptyness, and hopelessnes, (3) diminished interest or pleasure derived from activities that once brought pleasure to the individual, (4) significant weight loss (when not dieting) or weight gain due to a decrease/increase in eating habits, (5) insomnia or hypersomnia, (6) agitation or retardation, (7) fatigue, lethargy, or loss of energy, (8) persistent feeling of worthlessness or unwarranted guilt, (9) decreased ability to think or concentrate, and (10) continued repetitive thoughts of death, suicidal thoughts, and ideations without a plan to attempt suicide [2].

STATISTICS ON DEPRESSION

Depression is a mental illness that, according to the National Institute for Mental Health, globally affects 13% of all 12 to 18-year-old individuals [20]. In 2016, it was reported that roughly 13% of adolescents aged 12 to 17 had reported at least one major depressive episode and 9% of

adolescents reported a major depressive episode that led to severe impairment in their lives according to the Substance Abuse and Mental Health Services Administration [21]. Adolescent depression has been linked to an increase in suicide ideation and suicide attempts. The National Center for Injury Prevention & Control had also reported that there were a total of 1,300 suicides among adolescents from ages 12 to 17 in the United States [21]. Studies have repeatedly shown that roughly 90% of teenagers who have commited suicide were found to have had a mental illness based on their psychological autopsies, majority of these mental illnesses were found to be mood disorders [17]. Additionally, according to the Centers for Disease Control and Prevention, roughly 8.6% of high school students had reported at least one suicide attempt in 2015 [19]. According to the National Institute of Mental Health, the number of adolescents who reported a major depressive episode during 2015 (roughly 12.5%) was drastically higher than the previous 11 years [19].

Statistics for depression and suicidality also vary between gender and sexual identity. It has been found that adolescents who are transgender suffer from depression at a disproportionately higher rate in comparison to other groups. Some studies have indicated that there are as many as 40% of transgendered youth that have attempted suicide at some point in their life [15]. In addition to the drastically increased chance for attempted suicide among LGBTQ youth, there is also a high rate of estimated youth that are homeless. The percentage of homeless LGBTQ youth ranges from 30 to 45% [15]. Brief Symptom Inventory (BSI) was conducted by Bidell on LGBTQ youth in order to determine their Global Severity Index (GSI) scores [15]. Bidell found that 64.3% of LGBTQ youth had elevated GSI scores in comparison to non-LGBTQ youth; this suggested that LGBTQ youth had a higher susceptibility to mental health distress, symptoms, as well as higher scores in potential for depression, paranoid ideation, and

psychoticism [15]. Noell & Ochs and Whitbeck, Chen, Hoyt, Tyler, & Johnson found that homeless LGBTQ youth scored 9 - 20% higher than non-homeless in the possibility for suicidal ideation [15]. The statistics on individuals who contacted a national LGBTQ youth crisis service provider reported that homelessness was the most prevalent issue the LGBTQ youth faced. Roughly 32% of individuals had experienced homelessness, and a quarter of the 32% had experienced continual homelessness for the entirety of the previous month before calling the crisis service provider. Additionally, roughly 1 in 3 youth that have contacted the national LGBTQ crisis service program had experienced a lifetime of homelessness; high rates of homelessness was especially seen in transgender youth [15].

According to the National Institute of Mental Health, the number of adolescents who had reported a major depressive episode during 2015 were also significantly more likely to be female; 19.5% of females reported an episode in comparison to the 5.8% episode reports made by males [19]. The Center for Disease Control and Prevention reported that 20 - 40% of all girls reported feelings of depression; one national study in particular reported that 36% of all adolescent females had experienced sadness or hopelessness for nearly every day for two straight weeks in a row [2]. Additionally, the American Academy of Child and Adolescent Psychiatry and the Substance Abuse and Mental Health Services Administration have reported that females between the ages 12 and 15 have experienced a major depressive episode (5% at age 12 to 15% at age 15). By the age of 18, the percentage of reports from females experiencing at least one episode of Major Depressive Disorder increased to at least 20%, and roughly 10% of all the females have at that point attempted suicide [2]. A study conducted by Rohde, Beevers, Stice, & O'Neilin found that major and minor depression varied depending on not only age, but race and socioeconomic status as well [16]. They also found that white racial status and younger age were

closely related with increased feelings of worthlessness and suicidality during a Major Depressive Disorder episode [16]. Moreover, adolescents from racial/ethnic minority groups were at a higher risk than their counterparts to possibly experience or suffer from minor depression [16]. According to a study conducted by Delfabbro, Malvaso, Winefield, & Winefield, adolescent females were twice as likely than males to report occasional suicidal ideation, and 3.5 times more likely to report a suicide attempt at some point during their life [6].

GENDER DIFFERENCES AND HOW DEPRESSION CAN BE DISPLAYED

Depression among males is typically displayed differently and is less commonly reported when compared to females. Males and females during childhood have a 1:1 ratio of reported major depressive episodes; however, by age 13 the number of reported major depressive episodes changes to a 1:2 ratio [2]. To further understand gender disparity in depression, Tu, Li, & Cohen conducted a study where they tested whether the Polyvagal Theory would offer protective or non-protective factors against depression [20]. They tested two separate cardiac markers: RSA (measured as variations in heart rate across various breathing cycles) and RSAR (the reactivity of the RSA in response to different stimuli) [20]. It was discovered that higher baseline RSA emerged as a protective factor for females, but did not offer any protective benefits for males when it came to depression [20]. Fletcher et al. and Nederhof et al. found that RSAR indicated internalization of depressive symptoms particularly in males, which were not found in females [20]. There have been significant gender differences regarding suicidal behaviors, and it has been observed that males are typically more likely to die by suicide and have more serious health implications after a suicide attempt compared to females [12].

Risk Factors And Contributing Factors For Suicidality

There are many different and wide-ranging risk factors that result in an increased likelihood to die by suicide; these risk factors are not limited to depression or other mental illnesses. Through various forms of research, the possibilities and reasons for adolescent suicidality were found to be far more complex than simply a mental illness, rather it involves the interconnection of various unique attributing factors [6]. Familial risk factors for suicidality can entail the following attributes: (1) growing up in poverty, (2) family discord or chaos, (3) domestic violence in homes between parents or children, (4) parental divorce and separation, and (5) social environment that an individual is exposed to at a young age [6]. Individual risk factors also play a role in the possible increase in suicidality amongst adolescents. These risk factors can be described as an individual's psychological well-being, for example, if an individual suffers from a mood disorder, psychiatric morbidity of having multiple mental illnesses, or a general emotional strain [6].

General risk factors that were discovered to be related to increased suicidality by Evans, Marte, Betts, & Silliman were as follows: (1) poor coping skills, (2) family disputes, (3) stress, (4) substance abuse, (5) eating disorders, (6) loss of a loved one or significant other, (7) physical, emotional, mental, and sexual abuse, (8) death by suicide of a close relative/friend, (9) feelings of worthlessness and alienation by peers, and (10) depression, loneliness, and hopelessness [8]. Other studies discovered that being a victim of bullying at a young age could lead to increased suicidality [8]. Evans, Marte, Betts, & Silliman found through their study that there was an increased association between suicide risk/suicide ideation/general suicidality and youth violence [8]. Another study tested for the possible risk for suicidal thoughts and behaviors in adolescents who partook in direct self-injurious behaviors (D-SIB) [11]. It was found that a history of self-harm

was indicative of an increased risk for potential D-SIB in an adolescent's future [11]. Additionally, repeated self-injury, regardless of whether it was previous or recent, was a predictor for future suicide attempts [11]. Ribeiro, Franklin, Fox, et al. found that maintenance/continuous or late onset of D-SIB was associated with an increased likelihood of suicidal thoughts and behaviors in later adolescence [11]. However, it was also found that suicidal thoughts and behaviors did not increase in adolescents after direct self-injurious behaviors were terminated [11].

PROTECTIVE FACTORS AGAINST SUICIDALITY AND DEPRESSION

There are also protective factors against depression and suicidality, commonly found within communities outside of the individual's immediate family. A study conducted by Sibold, Edwards, Murray-Close, & Hudziak found that physical activity was associated with lower levels of sadness, suicidal ideation, and suicide attempts in adolescents from the United States [18]. It was found that adolescents that exercised more frequently (4 to 7 days per week) were found to have significantly lower levels of sadness, suicidal ideation, and suicide attempts than individuals who exercised less frequently. It was also found that being physically active (4 or more days per week) showed a direct negative relation in the likelihood of suicidal ideation and suicide attempts in bullied adolescents (approximately a 23% reduction). Studies have also evidenced that adolescent participation in religious and spiritual communities has led to an increase in improved mental health [12]. Psychosocial factors such as religion and spirituality can lead individuals onto trajectories involving reduced depression and suicidal behaviors [12]. Markstrom found that individuals who were religious and engaged in religious social networks had more hope and trust; conversely, Dervic, Oquendo, Grunebaum, Ellis, Burke, & Mann found that individuals with no religious affiliations were more likely to attempt suicide, or had a higher chance of having loved ones who had attempted suicide [12]. Religiosity can be a protective factor

for adolescents, especially younger females; however, the effects of religion were shown to become statistically insignificant in young adults [12].

KNOWLEDGE ABOUT DEPRESSION AND SUICIDALITY

Adolescent knowledge and understanding of depression and suicidality are important when it comes to reporting suicidal thoughts, ideation, risk, and depression among close family and friends. A study conducted by Reavley, Morgan, & Jorm found that adolescent females have more mental health literacy (MHL) than males and that MHL was directly associated with symptoms of mental illness [5]. Burns & Rapee found that females were more likely than males to recognize depression, assess a recovery time, and recommend acquiring help [5]. Horwitz, Storfer-Isser, Kerker, et al. found that in 2013, 66% of pediatricians reported they were not properly trained to treat adolescents who were suffering from depression [5].

COMORBIDITY OF OTHER ISSUES WITH DEPRESSION

It is common for an individual suffering from depression to also have another comorbid mental illness. Depression has been found to be present in 90% of suicides, even if it was comorbid with another mental illness [9]. Depression is comorbid with anxiety disorder and bipolar disorder in 70% and 15% of suicide cases, respectively [9]. Depression was also found to be comorbid with borderline personality disorder in 55% of adolescent patients who were suicidal [9]. Repeated suicide attempts were found to be more commonly correlated with patients who have been diagnosed with major depressive episodes, dysthymia, generalized anxiety, and panic disorder [9]. Suicidal ideation in adolescents with PTSD ranged between 30 - 80%, while suicide attempts in adolescents with PTSD ranged from 15 - 50% [14]. Adolescents with PTSD, where the rates of suicidal ideation were found to be roughly 70%, had rates of suicide attempts ranging

between 10 - 40% [14]. Chou, Liu, Hu, & Yen found through their study that 12.2% of adolescents who have ADHD also suffer from suicidal ideation or suicide attempts [3]. Chou, Liu, Hu, & Yen found that several factors such as individual older age, being a victim of bullying, and elevated depression levels increased suicidal intent in adolescents with ADHD [3]. It was also discovered that some ADHD-related symptoms imitate depressive symptoms in adolescents, such as distracted attention and impulsivity [3]. According to the Substance Abuse and Mental Health Services Administration, approximately 1.4% of adolescents in the United States had a comorbid substance-use disorder along with a major depressive episode [21]. Birmaher et al., Rohde, Lewinsohn, Klein, Seeley, & Gau found that roughly 40% of adolescents experiencing multiple major depressive episodes were frequently reported within adolescent depression studies [21]. Moreover, adolescents without past trauma reported lower depression severity scores in comparison to the adolescents who reported past trauma, and this was proved in a study that detailed that 53% of adolescents who sought out help for suicidality and depression also suffered from past trauma [21].

Overview Of Types Of Treatment For Depression

Treatment for depression has evolved throughout the years within the field of psychology. There were two majorly supported treatment methods for depression, and four other lesser-known but more specific treatment methods. The two major treatment methods are Cognitive Behavioral Therapy and Interpersonal Psychotherapy. Cognitive Behavioral Therapy has been described as a treatment that focuses on the immediate present and using skills that can be developed in order to treat depression in adolescents. This method views that depression is caused by how an adolescent interprets situations and events that occur around them [10]. Cognitive Behavioral Therapy can be categorized by identifying automatic thoughts and

distortions of cognition, creating thoughts that are counterintuitive to reality, activating behaviors in the patient, increasing involvement in activities that bring satisfaction and joy to the patient, and learning problem-solving skills [10]. It has been the most used psychotherapy for treating depression in adolescents; however, this method does not seem to have long-term advantages of remission, recovery, recurrence, or high functioning levels that are seen in the family or support therapy [10].

Interpersonal Psychotherapy, on the other hand, is one of the most recently developed psychotherapeutic treatments and interventions for individuals suffering from depression [10]. It was developed to originally be a time-limited treatment that focused on depression in non-bipolar outpatients [10]. The central idea of Interpersonal Psychotherapy for Adolescents is that clinical depression occurs in an interpersonal setting and that the commencing response to treatment is directly impacted by the interactions a patient has with others in their life [10].

A lesser-known and not as commonly used treatment for depression is family therapy, modified into attachment-based family therapy. Attachment-based family therapy is a brief manualized treatment that has been designed to address the specific needs of adolescents suffering from depression and their immediate families. It is based on the idea that disruptions in the family assist in creating chaos in critical roles such as development, maintenance, and the relapse of depression in children and adolescents [10]. Attachment-based family therapy has five main intervention tasks: relationships are reframed, alliances are established between the adolescent and the parent, failures are addressed within the attachment, competency is built, and is established. The reframing task is designed to build a foundation for the treatment by promoting improvement in familial relationships [10].

Young, Mufson, and Davies compared the efficacy of Interpersonal Psychotherapy for Adolescents to school counseling. A majority of students in high school are most likely to receive school counseling as the first form of treatment for their depression and is typically carried out by the school guidance counselor or social worker. The study conducted by Young, Mufson, and Davies reported that school counseling is not nearly as effective as Interpersonal Psychotherapy for Adolescents. While not as effective, it is important that school counseling is reinforced and carried out by trained professionals.

Young, Jones, Sbrilli, Benas, Spiro, Haimm, Gallop, Mufson, and Gillham conducted a comparison study of Interpersonal Psychotherapy for Adolescents and group counseling in a school setting [22]. Group counseling is another treatment for depression but has not been as commonly used as the two major therapies for depression treatment. The group counseling within the study conducted by Young, Jones, Sbrilli, et al. consisted of individual and group sessions that were led by school counselors [22]. The number of sessions of group counseling was made to match the frequency and duration of sessions conducted within the Interpersonal Psychotherapy for Adolescents group. Interestingly, it was discovered that both types of therapy effectively assisted in reducing depressive symptoms and increasing functioning for adolescents. Interpersonal Psychotherapy for Adolescents, and not group counseling, was found to be better in the short term; depressive symptoms dissipated after the 6-month follow-up session [22]. The group counseling participants, on the other hand, continued to have decreased reports of depressive symptoms and stable functioning for adolescents with depression well past the 6-month follow-up.

The most recently developed and investigated treatment for depression that was designed to target and assist Transgender Youth is the AFFIRMative Cognitive Behavioral Intervention

therapy. This therapy was developed via community-based research efforts to address the following: (1) a positive and affirmative approach to sexual and gender diversity, (2) recognition and awareness of sexual and gender identity specified stressors, (3) a youth-centered orientation that recognized and attended to unique experiences, trials, and tribulations that adolescents with sexual and gender minority identifications have experienced, and (4) utilize a Cognitive Behavioral Therapy within its own framework [1].

Unfortunately, sometimes therapy treatments do not work for individuals. O'Keeffe, Martin, Target, & Midgley established 3 different categories that would allocate adolescents who dropped out of their therapy treatment [13]. (1) Dissatisfied dropouts had reported that they stopped going to therapy because they found it was unhelpful for their situations. (2) Got-what-they-needed dropouts had reported that they felt they benefited from therapy, and thus, stopped going to it. (3) Troubled dropouts had been found to stop going to therapy because they had instability in their lives that were preventing them from continuing therapy. Interestingly, Wierzbicki & Pekarik and de Haan et al. found that therapy dropout can occur at any point during therapy and after any number of sessions [13]. Studies have found that there was a barrier to the treatment model; families that attend treatment for a child's conduct issues have a higher dropout chance [13]. Kazdin et al. found that it is generally assumed that dropout from therapy was thought to be an indicator of the failure of treatment by the therapist [13]. People who dropped out because they got-what-they-needed accounted for 42 - 45% of dropouts in BPI and CBT treatments, whereas dissatisfied dropouts accounted for 79% of STPP dropouts, and only 25% of BPI and 33% of CBT cases [13].

CONCLUSION

Depression is a mental illness that affects a significant portion of the world population. It is uninhibited by cultural boundaries, socioeconomic status, skin color, or even religion. Depression affects a disproportionate amount of adolescents, roughly 13% between the ages of 12 and 18, and has attributed to making suicide the third-leading cause of death in the United States [20,7]. However, depression is not the only direct cause of suicide among adolescents, nor is suicide singularly caused by depression among adolescents. Death by suicide is brought on by numerous reasons, such as depression comorbidity with another mental illness, socioeconomic status, familial risk factors, and body image issues. Depression and suicide are serious issues that plague adolescents around the world. The field of psychology has made an active effort to understand how the two relate to each other, and what can be done to decrease the severity of depression and the prevalence of suicide among adolescents.

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Meet The Authors

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Kevin authored the paper "The Nano-Mechanical Role of Melanin in Melanoma Cell" during the Spring 2020 semester under the guidance of Dr. William Gillis and Dr. Zulema Cabail. His other academic interests include learning about African American history. Beyond academics, Kevin enjoys working out, playing various sports and video games, and collecting vinyl records. He also volunteers at his family church in his spare time. Recently, due to the pandemic, Kevin has participated in several community service opportunities giving care packages to help those in need. His goal is to become a Nurse Practitioner.

Sameer Ahmed, B.S. Biological Science, Minor in Islamic Studies
Sameer is the author of the papers, "Finding Cosmic Strings with Black Hole-Pulsar Binaries" and "Investigating Estrogen Effects on Microglia and Glioblastoma Function" (Spring 2021). He worked under the guidance of Dr. Matthew Lippert and Dr. Jillian Nissen, respectively. His interest in Neuroscience and Astronomy played a key role in his passion for research and his pursuit of a career as a neurosurgeon. He is currently a research assistant in the following fields: theoretical physics, bioinformatics, and neuroimmunology. Sameer is a volunteer at NYU Langone Hospital and St. John Cantius Parish Garden for Senior Citizens; he also does shadowing at Stony Brook Hospital. He is the Co-Founder and Vice President of the Environmental Sustainability and Melittology club (ESM), the Marketing Head of *Cogito Ergo Sum: Honors Undergraduate Review*, and he is a member of the Honors College. In addition to his academic work, Sameer enjoys reading history-based novels and is passionate about global politics and economy.

Inayah Burton, B.S. Psychology
Inayah researched and wrote "Religiosity/Spirituality and Academic Performance in College Students" during the Fall 2020 semester under the guidance of Dr. Meesuk Ahn and, "Schizophrenia: An Assessment of High-risk Factors That May Lead to Diagnosis" during the Spring 2020 semester under the guidance of Dr. Lorenz Neuwirth. Her exposure to European History developed her interest in Religiosity/Spirituality research while her exposure to Introduction to Psychology helped develop her interest in psychiatric research. Inayah plans to enroll in an M.D./Ph.D. program in psychiatric medicine and focus on researching the diagnosis and treatments of psychopathologies. She is a member of the Honors College and CSTEP. Beyond academics, Inayah enjoys traveling, reading, playing video games, and crocheting. Her mother has been a big inspiration in her life as she respects her mother's love for learning, education, and helping others.

Stephanie Dagdagan, B.S. Psychology
Stephanie is the author of "COVID-19 and its Effect on Stress Levels and Academic Performance in College Students" (Spring 2020) under the guidance of Dr. Meesuk Ahn. Her experiences in Research, Design, and Analysis I & II heavily influenced her desire to get involved in research. Moreover, as a student taking classes during the pandemic, Stephanie felt very passionate about how COVID-19 would affect the academic performance of college students, which promoted her to pursue her research topic. She realized that students need more mental health resources to be readily available, and hopes her research helps to solve these issues. Outside of school, Stephanie is involved in social issues, volunteering, and activism. She also enjoys going to museums, travel-

ing, exercising, reading, and baking.

Simran Dhami, B.S. Chemistry
Simran authored "Synthesis of Two New Crown-Ether Cyclophanes for Measuring Cation-Aromatic Interactions In Solution" during the Spring 2020 semester under the guidance of Dr. Bright Emenike. His experiences in Organic Chemistry I & II made him excited about the field and he wanted to learn more, prompting him to get involved in research. Simran was a member of the Honors College and is the Co-founder of Women in Science & Engineering and Equality for All (WiSE & EA). In addition to his academic studies, Simran enjoys meeting new people and making connections with others. He loves baking, photography, and taking long walks with his dog. He also wants to learn dancing, cooking, and mixed martial arts. In the future, Simran will pursue a career in patient care, and he plans to become an optometrist.

Maryann Johnson, B.S. Biochemistry
Maryann researched and wrote two papers, "Investigating Estrogen Effects on Microglia and Glioblastoma Function" and "Study of Ganglioside GM3 Association to Insulin Resistance Reveals Potential Therapeutic Drug via Inhibition of GM3S" during the 2020-2021 academic year under the guidance of Dr. Jillian Nissen and Dr. Bright Emenike, respectively. Her interests in Biochemistry, Cell Biology, Cell and Molecular Neurobiology, and Anatomy and Physiology I & II inspired her to get involved in research. She is currently a research assistant for Dr. Jillian Nissen in the field of neuroimmunology. Her interests in medical biology and medicinal chemistry have influenced her decision to pursue a career in medicine. Maryann has volunteered in the Radiology Department at LIJ Northwell Hospital and she has volunteered as an OT aide at Parker Jewish Hospital. She is a member of the Honors College, Co-Editor-in-Chief for *Cogito Ergo Sum: Honors Undergraduate Review*, Secretary of the Environmental Sustainability and Melittology club (ESM), a Sunday School teacher, and is involved in her church choir. Beyond academics, she enjoys reading, playing the violin, watching Netflix, and playing badminton.

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Tania is the author of the papers, "Investigating Estrogen Effects on Microglia and Glioblastoma Function" and "Survival and Selective Reversibility of Latent HIV Infection in Microglial Cells." She completed these papers during the 2020-2021 academic year under the guidance of Dr. Jillian Nissen. Her experiences in Genetics, Cell and Molecular Neurobiology, Biochemistry, and Molecular Biology have influenced her involvement in research. She is currently a research assistant for Dr. Jillian Nissen in the field of neuroimmunology. Tania's experiences in the laboratories have inspired her to become a forensic scientist. She is a member of the Honors College, Co-Editor-in-Chief for *Cogito Ergo Sum: Honors Undergraduate Review*, and oversees Public Relations for the Environmental Sustainability and Melittology club (ESM). Tania has volunteered at Northport VA Medical Center and NYU Winthrop. Beyond academics, she enjoys playing badminton, watching Netflix, reading, and trying new restaurants.

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Mohammad authored two papers, "Finding Cosmic Strings with Black Hole-Pulsar Binaries" and "Investigating Estrogen Effects on Microglia and Glioblastoma Function," completed during the Spring 2021 semester under the guidance of Dr. Matthew Lippert and Dr. Jillian Nissen, respectively. His interest in Biology, Cell and Molecular Neurobiology, and General Physics has

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Brian P. Miller, B.A. Psychology

Brian wrote "Depression and Suicidality Among Adolescents" during the summer of 2020 under the guidance of Dr. Melissa Kiner. His interest in Abnormal Human Behavior and Developmental Psychology, and his personal experience as a crisis counselor and homeless shelter client advocate have influenced his involvement in this research. Brian has worked as a sex education advocate at SUNY Old Westbury, volunteered at the Boys and Girls Club of America, and shadowed a psychologist. His other interests include playing video games, going on adventures with his fiance, hanging out with friends, reading books, and playing with his cat. Brian's father has been a role model in his life who has always encouraged him to follow his dreams and never give up.

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Alfred is the author of "Enhancing the Liposome Uptake of Hydrophobic Porphyrin Using Cyclodextrin" completed during the Spring 2020 semester under the guidance of Dr. Ruomei Gao. His interest in chemistry has influenced his passion for research. Alfred also credits his Organic Chemistry Professor, Dr. Resch, for offering feedback and knowledge for his research. When he is not studying and performing research, Alfred enjoys watching movies in the theater, which he took for granted before the pandemic. He also has interests in trading/investing in the stock market, which has taught him patience and helped him focus on long-term goals. He aspires to become a Physician's Assistant, specializing in cardiology.

Advice from Authors to Students about Research at SUNY Old Westbury

Research is an excellent way to broaden the scope of your knowledge in your major field of study. Try to perform research early in your undergraduate education to have opportunities to get experience and build skills that will open up academic and career avenues in the future. SUNY Old Westbury provides a variety of research projects that you can correlate with the subjects that interest you. The key is to consult with your professors, read about their research, and then ask them if you can assist with their research. Let your professors know what specifically interests you about their research. Make sure they know that you are serious in your commitment to research, and in performing research with them. Be sure to thank professors for mentoring and guiding you. We wish you great success at SUNY Old Westbury!

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