About the Cover:

The COVID-19 pandemic has had a devastating impact on the world while changing the way society interacts with health and science. The images on the cover are four different electron micrographs of SARS-CoV-2 virus particles isolated from patients.

Courtesy: National Institute of Allergy and Infectious Diseases (www.flickr.com/photos/niaid/)
# Table of Contents

Starch Inclusion Complexes with C18 Fatty Acids of Different Degrees of Unsaturation  
Isabella Gladden………………………………………………………………………………………………2

A Review of Elasmobranch Systematics: Speciation, Phylogeny, and Implications for Conservation  
Patrick Lewis…………………………………………………………………………………………………7

The Relationship between Hospital Patient Safety Culture and Patient Satisfaction: A Systematic Literature Review  
Joel Bass, Christian Fields, Lauryn Vaughn, Amy Ratley, Fallon Davis………………………………………20

Medical Cannabis in the Treatment of Parkinson’s Disease  
Colin Begley…………………………………………………………………………………………………29

Examining the Relationship Between Cortisol Levels, Environmental Factors, and Developmental Outcomes in Young Children  
Amber McCranie……………………………………………………………………………………………..40

Differences in Physiologic Measures of Infant Swallowing Function for Swallows with Varying Degrees of Airway Invasion  
Sarah G. Trulove…………………………………………………………………………………………….47

Oral Retinoic Acid Treatments Modulate Adipose Tissue Development of Neonatal Offspring of Sprague-Dawley Rats Consuming a High Fat Diet  
Heleena Haberer ..................................................................................................................62

Interview with Dr. John Higginbotham  
JOSHUA………………………………………………………………………………………………………70

Interview with Dr. Russell J. Mumper  
JOSHUA………………………………………………………………………………………………………73

Interview with Dr. Sharlene Newman  
JOSHUA………………………………………………………………………………………………………75
Starch Inclusion Complexes with C18 Fatty Acids of Different Degrees of Unsaturation

Isabella Gladden¹, Jingyi Zhou², Lingyan Kong², Alegna Reyes³, Lingyan Kong²*¹

Faculty Mentor: Lingyan Kong

¹Department of Mechanical Engineering, The University of Alabama, Tuscaloosa, AL 35487
²Department of Human Nutrition and Hospitality Management, The University of Alabama, Tuscaloosa, AL 35487
³Department of Management, The University of Alabama, Tuscaloosa, AL 35487
*Corresponding authors

Starch, especially its amylose component, is known to form inclusion complexes with a variety of small molecules. Fatty acid complexations formed with starch have attracted much attention as a type of novel resistant starch. The structure of the lipid guest affects its complexation and properties and thus its resistance to enzymatic digestion. The aim of the present study was to investigate the complexation ability of high amylose maize starch (HAMs) inclusion complexes with C18 fatty acids of various double bond numbers and positions, including octadecanoic (stearic, SA), (Z)-octadec-9-enoic (oleic, OA), (E)-octadec-9-enoic (elaidic, EA), (9Z,12Z)-octadeca-9,12-dienoic (linoleic, LA), (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (α-linolenic, ALA), and conjugated linoleic acids (CLA). The formation of inclusion complexes was examined by complementary techniques including differential scanning calorimetry, X-ray diffraction, and Fourier transform infrared spectroscopy. The results showed that all C18 fatty acids formed inclusion complexes with high amylose maize starch. Thermal stability of the inclusion complex decreased with the degree of unsaturation of the fatty acids.

Introduction

Starch is one of the most important macronutrients, serving as the main reserve of carbohydrates in the human body. The source and chemical structure of starch determines its digestion by the body, leading to varying rates of glucose absorption. Based on the kinetics of the starch’s digestion, starch can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [2]. When starch is composed mostly of RDS, the starch is quickly digested into glucose and can cause postprandial hyperglycemia. This condition can be detrimental to those with type 2 diabetes, heart disease, and obesity. Alternatively, when starch is composed of mostly SDS and RS, the starch is digested at a slower rate and leads to a steadier increase in the blood glucose level. Additionally, RS has been shown to have a positive impact on the gut microbiome through its fermentation and resulting production of short-chain fatty acids.

In the presence of certain small molecules, starch, specifically amylose, has been shown to form starch-guest inclusion complexes [7]. Due to its linear, unbranched nature, amylose is able to form a helical structure, with a hydrophilic exterior and hydrophobic cavity. The interactions between the amylose and the guest molecule lead to chemical and structural changes that can affect the digestibility of the starch.

Fatty acids and their esters are the most widely-known guest compounds. In the presence of amylose, the aliphatic chain of the fatty acid is surrounded by the coils of the amylose with the polar head of the guest outside of the cavity [5]. Fatty acids are common components of more complex lipids and are composed of a carboxyl group with hydrocarbon chains of...
varying lengths. In addition to the fatty acids having various carbon chain lengths, the structure of the fatty acid can vary based on the number and location of double bonds in the chain. Another layer of complexity of the double bonds is whether they are in the cis- or trans-configuration. These combined structural changes can lead to the fatty acid chain curving, which may influence its complexation ability with amylose and the microstructural and physicochemical properties of the inclusion complex [4,8].

In this study, various C18 fatty acids including octadecanoic (stearic, SA), (Z)-octadec-9-enoic (oleic, OA), (E)-octadec-9-enoic (elaidic, EA), (9Z,12Z)-octadeca-9,12-dienoic (linoleic, LA), (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (α-linolenic, ALA), and conjugated linoleic acids (CLA) were used as the guest molecule. The ability of the fatty acids to successfully form inclusion complexes as well as the subsequent physicochemical properties of the inclusion complexes were investigated.

Materials and Methods

Materials

High amylose maize starch (HAMS; Hylon VII) was provided by Ingredion (Bridgewater, NJ, USA). Stearic acid (S0163) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Elaidic acid (A14832) was purchased from Alfa Aesar Inc. (Ward Hill, MA, USA). Linoleic acid (436305), oleic acid (75090), conjugated linoleic acid (O5507), and α-linolenic acids (L2376) were purchased from MilliporeSigma (Burlington, MA, USA). Dimethyl sulfoxide (DMSO), Drierite™ desiccant, and ethanol were purchased from VWR International (Radnor, PA, USA).

Preparation of starch inclusion complex

Starch inclusion complexes were prepared using the DMSO method described by Kong, Bhosale, and Ziegler [3] with some modifications. HAMS (500 mg) was dissolved in 10 mL of 95% (v/v) DMSO, and the solution was heated in a 90°C water bath with constant stirring for 30 minutes. The guest compound was prepared by dissolving 50 mg of the fatty acid sample in 1 mL of 95% (v/v) DMSO. The guest solution was added to the starch aqueous solution and kept at 90°C in the water bath with constant stirring for an additional 15 minutes. Deionized water (25 mL) was heated to 90°C in another water bath and added to the HAMS-guest solution. The mixture was kept at 90°C with constant stirring for another 15 minutes. The solution was removed from the heat and allowed to cool to room temperature for 24 hours. To recover the precipitate, the solution was centrifuged (3000 rpm, 15 min) and washed with 50% (v/v) aqueous ethanol three times. The solution was centrifuged (3000 rpm, 10 min) between each washing. The resulting mixture was transferred to an aluminum pan with a small amount of pure ethanol and allowed to fully dry in a desiccator containing Drierite™ as the desiccant. The dried mixture was ground into a fine powder to be analyzed.

Differential scanning calorimeter (DSC)

Approximately 2 mg of dry sample was weighed using a Mettler-Toledo XP2U ultramicrobalance (Mettler-Toledo International Inc., Columbus, OH, USA) into a large-volume stainless steel DSC pan (Perkin-Elmer Instruments, Norwalk, CT, USA). Deionized water was added to obtain a 10% (w/v) dispersion. The DSC pan was hermetically sealed. Using an empty pan as the reference, the pans were equilibrated to 10°C and then heated to 120°C at 5°C/min in a Discovery DSC 250 DSC (TA Instruments, New Castle, DE, USA).

Wide-angle X-ray diffraction (XRD)

Wide-angle X-ray diffraction patterns were obtained with a Bruker D8 Discover X-ray diffractometer (Bruker Corporation, Billerica, MA, USA) for HAMS inclusion complex samples. The ground samples were exposed to Co Kα radiation (1.79 Å) and scanned over the range 4° to 30° (2θ). Data obtained using the Co Kα radiation source were converted to Cu Kα radiation based 20 values.

Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared (FTIR) spectra of inclusion complex samples were
obtained using a PerkinElmer Spectrum 100 FTIR spectrometer (PerkinElmer Inc., Waltham, MA, USA), with an attenuated total reflection (ATR) accessory. The spectra were recorded in the wavelength region of 650 to 4000 cm\(^{-1}\), with an average of 32 scans at a resolution of 4 cm\(^{-1}\).

**Statistical analysis**

Results were recorded as the means ± standard deviation, and significant differences between groups were tested using one-way analysis of variance (ANOVA) and Fisher’s Least Significant Difference (LSD) test. Statistical analysis was conducted using SPSS version 19.0 software (SPSS, Inc., Chicago, IL, USA). The letters a, b, and c indicate statistically significant differences, \( p < 0.05 \) (a>b>c).

**Results and Discussion**

**Inclusion complex formation**

Complementary techniques including DSC, XRD, and FTIR were used to confirm the formation of inclusion complexes. On a DSC thermogram, inclusion complexes, if formed, would show an endothermic peak during heating and an exothermic peak during cooling, indicating the dissociation and reassociation of the inclusion complexes formed [1]. Among the prepared samples in this study, visible endothermic peaks were observed for all samples of precipitated HAMS with the guest C18 fatty acids (Figure 1A).

Endothermic peaks were observed at around 96°C, 94°C, 92°C, 83°C, 81°C, and 90°C for precipitated HAMS-SA, EA, OA, LA, ALA, and CLA, respectively. These dissociation temperatures occurred within the range of 80 to 120°C [4], which is indicative of the formation of inclusion complexes between the starch and guest compounds. The additional endothermic peak observed at around 68°C for the precipitated HAMS-SA was due to the melting of uncomplexed stearic acid (Figure 1B). These results together suggested that the thermal stability decreased as the degree of unsaturation of fatty acids increased. SA, which required more energy during the dissociation and association process, had the highest stability, and ALA, which required less energy during the same process, had the lowest stability.

In addition, although OA and EA both obtained the same unsaturation degree, the \( \text{trans} \) structure of EA increased its thermal stability compared to the \( \text{cis} \) structure of OA. Such a phenomenon could be explained by the fact that the \( \text{cis} \) configuration disrupted the linear shape of the fatty acid while the \( \text{trans} \) structure resembled the linear structure of saturated fatty acids. Furthermore, when comparing LA to CLA, the thermal stability of the inclusion complex increased when the structure became conjugated due to the presence of \( \text{trans} \) double bonds in the conjugated dienes.
Figure 1. DSC thermograms of (A) pure C18 fatty acids and (B) precipitated high amylose maize starch samples with (a) stearic acid (SA), (b) elaidic acid (EA), (c) oleic acid (OA), (d) linoleic acid (LA), (e) α-linolenic acid (ALA), and (f) conjugated linoleic acid (CLA).

Figure 2. XRD patterns of precipitated high amylose maize starch (HAMS) samples with stearic acid (SA), oleic acid (OA), elaidic acid (EA), linoleic acid (LA), α-linolenic acid (ALA), and conjugated linoleic acid (CLA).

Figure 3. FTIR patterns of precipitated high amylose maize starch (HAMS) samples with stearic acid (SA), oleic acid (OA), elaidic acid (EA), linoleic acid (LA), α-linolenic acid (ALA), and conjugated linoleic acid (CLA).
XRD patterns (Figure 2) of the precipitated HAMS and guest compounds provided evidence of the formation of starch-guest inclusion complexes through characteristic peaks observed at various angles. All of the HAMS-fatty acid samples exhibited V-type characteristic peaks at 13° and 20°, which is indicative of the formation of V₆-type structure [4]. The XRD patterns supported the DSC thermogram results, confirming that the C18 fatty acids successfully formed inclusion complexes with HAMS.

FTIR (Figure 3) is a useful technique used to identify functional groups that are present in a sample. It can be used to observe if the guest compound was successfully encapsulated in the helical amylose. All of the HAMS-fatty acid samples exhibited peaks at around 2850 cm⁻¹ and 2920 cm⁻¹, with the HAMS-SA sample having the sharpest peak. These correspond to the stretching vibration of the CH₂ groups on the fatty acid chain [6]. The decrease in the intensity of the peaks as the number of double bonds increased could be due to fewer guest molecules being encapsulated or fewer CH₂ groups in the aliphatic chain.

Conclusion
The ability of HAMS to form inclusion complexes with various C18 fatty acids was observed through various techniques such as DSC, XRD, and FTIR. All fatty acid samples were able to form inclusion complexes, with stearic acid forming inclusion complexes most effectively. Stearic acid is a saturated fatty acid and has a highly linear structure, allowing the turns of the amylose helix to better wrap around the aliphatic chain. As the fatty acids became more unsaturated, they became less effective at forming inclusion complexes due to the change in chemical structure. The ability of the fatty acids to form inclusion complexes could allow for the inclusion complex to protect the fatty acids, as well as alter the digestibility. Additional research on the effect on the digestion profile could yield significant results on how saturation affects the ability of the inclusion complex to resist digestion.

Declaration of Competing Interest
The authors declare no conflict of interest.

Acknowledgments
The authors would like to thank Dr. Jason Bara, Associate Professor in the Department of Chemical and Biological Engineering, and his graduate student, Shuai Qian, for their help with the FTIR spectrometer. The Randall Research Scholars Program of the University of Alabama is acknowledged for sponsoring I. Gladden as an undergraduate researcher.

References
A Review of Elasmobranch Systematics: Speciation, Phylogeny, and Implications for Conservation

Patrick Lewis

Faculty mentor: Dr. Jason Pienaar

Department of Biological Sciences, the University of Alabama, Tuscaloosa, AL, 35487

Four hundred million years of existence and abundant environmental variation have allowed ample opportunity for elasmobranchs (sharks, rays, and guitarfish) to evolve and speciate. As a result, they number over a thousand species, inhabit all the world’s oceans, and occupy a wide variety of niches. While basic ecology and life history of the most visible species are known, elasmobranch evolutionary history is direly understudied. This review offers insight into the speciation and phylogeny of sharks, rays, and guitarfish worldwide and examines current hypotheses, as well as the morphological and phylogenetic studies from which these hypotheses are derived and calibrated. It also investigates the occurrence of cryptic species and mechanisms underlying range expansion. Finally, it advocates for understanding how these processes, complicated by anthropogenic-derived effects on the environment, relate to conservation efforts. Ultimately, the review recommends continued study into both morphology and molecular phylogeny of elasmobranchs, each informing the other. Understanding elasmobranch speciation, biodiversity, and evolutionary history will help improve appreciation of extant species, adjust fishery conservation policy to accommodate them, and preserve vital marine ecosystems and ecosystem services.

Introduction

The subclass Elasmobranchii, of the class Chondrichthyes (“cartilaginous fish”), includes all species of sharks, rays, guitarfish, skates, and sawfish [4]. The hypothesized ancestors of the species seen today first appeared in the fossil record between the early Ordovician and the early Devonian Periods and came into their own as a class after the mass extinction of many prehistoric fish species at the end of the Devonian. The modern species evolved in the late Paleozoic era, proliferating throughout the Jurassic and Cretaceous Periods [2]. The elasmobranchs have since evolved into over a thousand morphologically diverse species occupying a variety of niches [9,24]. While white sharks, tiger sharks, and other charismatic species found in our aquariums and beaches get much of the attention and targeted conservation efforts, the ecological observations for these charismatic species are not representative of the hundreds of other, highly diverse species of elasmobranchs [14]. The variety of niches that these lesser-known species occupy necessitates a broader understanding of elasmobranch ecology to understand their roles in marine ecosystems at large. Likewise, a full comprehension of this ecology requires an appreciation of their evolution, which is often lacking in current scientific studies [16,33]. Research into the evolutionary history of elasmobranchs can yield insight into their diversification and provide conservationists foresight into how contemporary environmental changes will affect species and ecosystems in the future. In addition, a better understanding of their evolutionary history can also uncover cryptic species, leading to more accurate estimates of current biodiversity that can inform conservation efforts. Neglecting evolutionary history when crafting fishery policies that manage populations of exploited species for harvest by humans risks taking an incomplete view of the target species’ ecological breadth, inviting the destabilization of target fisheries and ecosystems [18]. This paper will review the evolutionary history and current evolutionary trends of elasmobranchs and how these can inform targeted conservation efforts for elasmobranchs on a global scale.
Species Concepts and Speciation Mechanisms in Elasmobranchs

Speciation, as defined by Seehausen, et al. [30], is “the origin of reproductive barriers among populations that permit the maintenance of genetic and phenotypic distinctiveness of these populations in geographical proximity” (p. 176). These reproductive barriers fall under one of three reproductive isolation mechanisms: prezygotic, intrinsic postzygotic, and extrinsic postzygotic. When analyzing specimens in practice, three schools of thought on how to designate species are commonly used: the biological, morphological, and phylogenetic species concepts. For definitions and explanations of these terms, see Figure 1.

**Speciation**

- **Prezygotic**: factors that arise before fertilization (though not necessarily before mating) that prevent fertilization.
  - Mate selection
  - Habitat preference
  - Disadvantageous timing

- **Intrinsic postzygotic**: simple “genetic incompatibilities” that occur regardless of environment.

- **Extrinsic postzygotic**: “Divergent ecological or sexual selection,” or all factors that negatively affect the fitness of the offspring.
  - Bateson–Dobzhansky–Muller incompatibilities (DMIs): fitness of hybrids is reduced via epistatic interactions between alleles at multiple loci.
  - Typically lead to inviable offspring, but do not affect the fitness of the parents.
  - Intrinsic factors may build upon one another.

**Sympatric speciation**: occurs within the same geographic region.

**Allopatric speciation**: occurs when species become separated by a geographic barrier.

**Biological Species Concept**: members of separate populations that interbreed and produce viable offspring

**Morphological Species Concept**: species are distinguished based on series of unique characteristics

**Phylogenetic Species Concept**: species are distinguished by similarity to one or more common ancestors

---

**Figure 1**: Speciation Concepts and Mechanisms adapted from Benton [2], Futuyma and Kirkpatrick [15], and Seehausen, et al.[30].
This review will focus primarily on prezygotic isolation mechanisms, specifically allopatric speciation, and the potential influences of anthropogenic activity on this process. For evolutionary analyses, this review focuses on studies employing the morphological and phylogenetic species concepts that paleontologists prefer because the biological species concept cannot be applied to fossils [2].

Study of Evolutionary History

The extensive evolutionary history of elasmobranchs has been studied for centuries [31]. Typically, these studies rely on the morphological examination of fossils and specimens, but scientists are increasingly relying on genetic analyses of extant species to gain insights into speciation and phylogeny. The combination of these two types of studies, aided by advances in computational technology, have yielded new insights into elasmobranch evolution.

Morphological Examination of Fossils and Specimens

Much like extant species, remains of extinct species can be analyzed and ordered by continuous, gradual morphological changes, allowing paleontologists to track the evolution of morphology over time. Unfortunately, most potential fossils have been weathered away over time, and cartilaginous fossils are too fragile to survive in great numbers for long, leaving few preserved skeletal remains to study. Many early species of sharks, particularly those from the Mesozoic and Cenozoic, are characterized only by fossilized teeth [1,11,22,23,34]. Such teeth are abundant in places like Alabama, where the Coastal Plain region was once covered by a shallow sea. Ancient marine fossils have remained preserved over millions of years in chalk, sand, and clay formations throughout Alabama, gradually becoming exposed through erosion by the state’s thousands of miles of waterways [19,21,22]. Specimens of more complete fossils, from jaws to complete skeletons, and even preserved muscle in rare cases, are crucial to understanding major transitions in the evolutionary history of elasmobranchs and provide a wealth of data points to track morphological changes over time [1,6,12,23,34]. “Fossils” from these cartilaginous species typically amount only to impressions in the surrounding rock (save for the calcified vertebrae and mandibles). Typically, identifications and evaluations of cartilaginous skeletons are derived from teeth or fragments of specimens, or through computed tomography (CT) analyses of fossil impressions, rather than complete specimens [1,6,23]. When they are compared to the known age of the surrounding rock layer, or strata, from which the fossil was extracted, paleontologists have been able to glean insights into evolutionary trends, trace relatives and distant ancestors of known species, and describe new species. In addition, paleontologists have developed hypotheses concerning migration and speciation patterns of ancient elasmobranchs over time, particularly in response to changes in the environment, by examining the surrounding strata for other fossils or sediments indicative of past habitats or climates [1,2,21,22,34]. Today, current elasmobranch specimens are routinely analyzed for minute morphological variations. Museums, literature, and collections from around the world offer a wealth of specimens and data from across deep time. Modern computers and X-ray machines offer a more precise analysis of morphological differences (mandible suspension, vertebrae count, etc.) and can facilitate the estimation of phylogenetic relationships, bringing traditional morphological analysis into the technological era [4,8,28,32].

Genetic and Genomic Analysis

While morphology remains a crucial consideration in these studies, DNA sequence data derived from the genomes of extant elasmobranchs have yielded rich new understandings of the natural history of these species. The goal of phylogenetic analyses is to determine the relationships between species within a group and their location in evolutionary history relative to other taxa [32].

Many contemporary phylogenetic studies examine sequence data from a few so-called phylogenetically informative mitochondrial and nuclear genes from liver, blood, or muscle cell DNA of sampled individuals. To resolve recent speciation events and geographically isolated variants, the
The relatively fast-evolving mitochondrial NADH2 marker is the most targeted gene sequence for analysis. The slower-evolving cytochrome oxidase c 1 marker, nuclear genes such as the nuclear-encoded LDHA6 locus, and mitochondrial DNA control regions provide resolution over longer time spans and are often used in combination with NADH2 sequence analysis to provide resolution for phylogenies that include both older and more recent speciation events [3,10,17,24,27,28,33,35]. Going beyond individual or groups of genes, whole genome mapping isolates, sequences, assembles, and annotates draft genome sequences of species from sampled individuals. While few whole elasmobranch genomes have been sequenced and assembled, phylogenomic analysis and comparison of these genomes to those of other vertebrate species have yielded key insights into elasmobranch and vertebral evolution, confirmed the phylogenetic position of elasmobranchs within the vertebrate phylogeny, and provided clues regarding the ecology of inaccessible, hard to study species [16].

For all phylogenetic comparative studies, new genes or genomes can be compared with existing ones and arranged via computer into maximum parsimony and likelihood phylogenies that depict the simplest, most likely evolutionary relationships between species. These phylogenies show new gene orthologue groupings that can be used to determine the positions of elasmobranchs within the tree of life [10,16,33,35]. Future phylogenomic studies, especially when combined with the latest morphological studies, could shed light on previously hidden aspects of elasmobranch evolutionary history, correct mistakes in species classifications and positions in the elasmobranch phylogeny, provide insights into the environmental catalysts for speciation, inform scientists about the ecology of rare and inaccessible species, and provide missing pieces to the puzzle of early vertebrate evolution [10,16].

Current Evolutionary and Phylogenetic Theory

The most recent predecessors to the elasmobranchs have been dated to the Upper Ordovician to Silurian Periods [2,7,29]. While the most primitive species possessed bony skeletons, the first instance of a truly cartilaginous skeleton, indicating the origins of the class Chondrichthyes, appeared in the early Devonian Period. This skeleton was made of calcified cartilage, giving it a degree of integrity [2,7]. The Chondrichthyes diverged from the Osteichthyes (“bony fishes”) and other jawed vertebrates, which had already diverged from jawless vertebrates such as lampreys millions of years before, and persisted through the Devonian [2,16]. The extinction of other species opened up niches that allowed both the Chondrichthyes and Osteichthyes to radiate in diversity through the late Paleozoic Era. Modern elasmobranchs and a subset of extinct relatives, known as neoselachians, evolved in the late Devonian and Carboniferous Periods, radiating during the late Triassic Period through the Mesozoic Era, a process started in the early Jurassic Period [2,11,34]. By the end of the Cretaceous Period, between 42 and 49 distinct families had evolved, comprising the families that are seen in modern times [2,9]. This radiation corresponded with simultaneous radiations in sea reptiles, such as plesiosaurs, and bony fish. It is hypothesized that this radiation in bony fish evolution introduced new food sources, allowing these competing predators to thrive and evolve new morphologies, hunting strategies, and species. By this point, early elasmobranchs had evolved true cartilaginous skeletons, making complete fossilized skeletons difficult to acquire. Therefore, many classifications of species from that time to present day are based on fossilized teeth. The KT Event, the mass extinction that doomed the dinosaurs at the end of the Mesozoic Era 65 million years ago, also eliminated most elasmobranch diversity. The species that survived the mass extinction event have mostly persisted to this day [2].

Today’s modern sharks, rays, and guitarfish can generally be divided into three to four orders, each consisting of a few families. Each family has a distinct body plan and ecology relative to the other families or groups [2]. The modern neoselachians are divided as follows:

The Galeomorphs, a clade of 250 species, consists of the
Heterodontiformes (the bullhead sharks), the Orectolobiformes (the carpet sharks), the Lamniformes (the mackerel sharks), and the Carcharhiniformes (the requiem and hammerhead sharks). The Galeomorphs occupy a wide breadth of habitats (typically littoral zones, although the Lamniformes are more pelagic), feeding modes, and niches and include many of the more charismatic species of shark. The Galeomorphs, as they are known today, originated around 273 million years ago (Mya). The Heterodontiformes split from the Galeomorphs around 250 Mya and radiated relatively recently, around 47 Mya. The Orectolobiformes, Lamniformes, and Carcharhiniformes all date back to Jurassic origins, specifically 166 Mya, 162 Mya, and 179 Mya, respectively. These species radiated in the late Jurassic and early Cenozoic, which is consistent with the radiation of bony fish and other marine predators [2,4,8,32-35]. While the bulk of Carcharhiniform diversity radiated into distinct species about 63 Mya, the Orectolobiformes of Australia/New Guinea radiated as recently as the Miocene, around 25 Mya, due to a rapidly changing environment caused by glaciation and global cooling that created barriers to dispersal [2,10].

The Squalimorphs, a much older clade than the Galeomorphs, include the Hexanchiformes (frilled and cow sharks), the Pristiophoriformes (the sawsharks), and the Squatiniformes (the dogfish). Squalimorphs tend to occupy colder, deeper waters and benthic habitats. As such, relatively little is known about them. The Hexanchiformes, Pristiophoriformes, and Squatiniformes all originated in the early Mesozoic (238 Mya, 200 Mya, and 216 Mya, respectively). The bulk of the diversity within this clade originated around the late Cretaceous and Paleogene Periods [2,4,8,32-35].

The Squatiniformes (the angel sharks and the monkfish) have only recently been proposed as distinct from the rest of the Squalimorphs. Their “raylike morphology” and similarity in niches to the Squalimorphs have made their placement in elasmobranch phylogeny controversial for over a century. For example, while Shirai’s [32] morphological analysis indicates that the Squatiniformes occupy their own order, the phylogenetic study conducted by Vélez-Zuazo, et al. [35] concludes that Squatiniformes originate from the larger Squalimorph order. The Squatiniformes have similar evolutionary histories to the Squalimorphs, originating in the early Mesozoic, about 200 Mya [2,4,8,33,34].

The Squatiniformes have similar evolutionary histories to the Squalimorphs, originating in the early Mesozoic, about 200 Mya [2,4,8,33,34]. The Batoids, 500 species in all, include the skates, rays, and guitarfish and are considered separate from the Galeomorphs, Squalimorphs, and Squatiniformes. With their mostly or completely flattened bodies, the Batoids are specialized for life on the ocean floor and typically consume benthic crustaceans. The Batoids diverged from the neoselachians sometime in the Devonian or Lower Carboniferous, around 364 Mya [2,4,8,32-35].

The phylogenies described above, from Vélez-Zuazo, et al. and Compagno, as well as representative species from the Galeomorphs, Squalimorphs, and Squatiniformes, can be seen in Figures 2, 3, and 4, respectively.
Figure 2: The elasmobranch phylogenetic tree, depicting evolutionary relationships as proposed by Vélez-Zuazo, et al. [35]. This figure is derived from genetic analyses. Notice how Vélez-Zuazo, et al. includes the Squatiniformes in the Squalimorpha order, differing from Compagno.
Figure 3: The elasmobranch phylogenetic tree, depicting evolutionary relationships as proposed by Compagno [8] and adapted from de Carvalho [4]. These relationships are derived from morphological analyses. Notice how Compagno separates the Squatiniformes into a separate order (here called Squatinomorphii), differing from Vélez-Zuazo, et al.

Figure 4: Photos and caption from Vélez-Zuazo, et al. [35]. “Representatives of the eight shark orders. (A) Carcharhiniformes (Blacktip reef shark, Carcharhinus melanopterus, photo credit: David Burdick); (B) Lamniformes (Great white shark, Carcharodon carcharias, photo credit: Terry Goss); (C) Orectolobiformes (Whale shark, Rhincodon typus, photo credit: Zac Wolf); (D) Heterodontiformes (Crested bullhead shark, Heterodontus galeatus, photo credit: Taso Viglas); (E) Squaliformes (Spiny dogfish, Squalus acanthias, photo credit: Doug Costa); (F) Squatiniformes (Angelshark, Squatina squatina, photo credit: Philippe Guillaume); (G) Hexanchiformes (Broadnose sevengill shark, Notorynchus cepedianus, photo credit: Jose Maria Perez Nuñez) and (H) Pristiophoriformes (Longnose sawshark, Pristiophorus cirratus, photo credit: Kevin Widjaja). Photos of B, C, and H are licensed under Creative Commons Attribution-Share Alike 2.5 Generic, and photos of D, F, and G are licensed under Creative Commons Attribution-Share Alike 2.0. The rest of the photos are from public domain.”
Present-Day Studies of Elasmobranch Speciation and Phylogenetics

New technologies have opened more avenues of inquiry into elasmobranch natural history and biodiversity. Today’s morphological and phylogenetic studies, utilizing the latest computing technologies, give scientists new insights into the ecology and phylogeny of elasmobranchs.

Improvements to Estimated Phylogenetic Relationships

Although current studies generally confirm or elaborate on the previously-proposed lineages, some mistakes have been found and rectified. Corrigan and Beheregaray [10] were able to modify the lineage of wobbegong sharks (Family Orectolobidae) in the southwest Pacific Ocean near Australia and propose relatively recent radiation in speciation within Orectolobidae. Corrigan and Beheregaray propose that speciation within the family in the area started with massive environmental change in the Miocene Period and continued into the Pliocene Period. Sorenson, et al. [33] estimated divergence times from genetic analyses of sampled elasmobranchs for 268 species, representing all orders of sharks globally, and compared data with habitat preference of the species collected. Sorenson, et al. confirmed the split in evolutionary trajectory between Squalimorpha and Galeomorpha, as well as within Galeomorpha, due to niche expansion spatially across habitats. However, an analysis of the most common mitochondrial and nuclear genes of 229 unique species of shark by Vélez-Zuazo, et al. [35] proposed revisions to lineages at the order and family level, creating some controversy. Vélez-Zuazo, et al. disagrees with contemporaries who believe the Squatiniformes are a separate order from Squalimorpha and instead suggests they are a family within Squalimorpha. It should be noted that, with 229 different species represented, each with varying availability of genes to be used for analysis, that the data needed is considered “incomplete” and demands more in-depth analysis. However, Vélez-Zuazo, et al. were able to propose avenues for further study, particularly within the order Squalimorpha.

Uncovering Cryptic Species

While fishery study and policy can be tailored around known biodiversity, they can neglect subsets of unknown species categorized within known ones. These “cryptic species” typically resemble known species morphologically but are genetically divergent [20]. For example, a study in the Arabian Sea, where research into biodiversity and fisheries ecology is lacking, involving samples from 1,487 specimens examined mitochondrial NADH2 markers from DNA samples for phylogenetic analyses. It yielded the possible existence of four cryptic species of guitarfish and presented evidence that previous range studies concerning native species of rays were flawed and deserved revisiting [17]. A more general study analyzing 4,283 samples from about 574 known species across the world indicated the presence of 79 undescribed cryptic species and pointed to areas that deserve higher scrutiny: the Red and Arabian Seas, due to the lack of previous research in the area, as well as around Southeast Asia and Australia, known hotspots for unique species [24]. However, Henderson, et al. [17] note that genetic analyses are of limited use without morphological comparison. In one instance, two morphologically dissimilar species were determined to be the same species phylogenetically through genetic analysis. Henderson, et al. also note, concurring with Naylor, et al. [24], that mitochondrial NADH2 analysis is not specific enough to depict instances of hybridization or very recent radiations. Earlier, Quattro, et al. (2006) [28] surveyed scalloped hammerheads (Sphyrna lewini) across the world, utilizing cytochrome oxidase c 1 marker assays in conjunction with vertebral (morphological) analysis. Quattro, et al. (2006) confirmed divergence between Atlantic and Indo-Pacific populations and indicated reproductive isolation amongst western Atlantic populations, signifying the presence of cryptic scalloped hammerhead species. Quattro, et al. (2013) [27] tentatively confirmed this hypothesis and described the presence of a new species in western Atlantic waters. Using 54 collected specimens, Quattro, et al. (2013) tested hypotheses derived from mitochondrial gene analysis in Quattro, et al.
(2006) with morphological characteristics and further mitochondrial and nuclear gene analysis. However, neither study could identify specific speciation mechanisms without further study on this new species’ abundance, ecology, and range.

Insights into Evolution, Ecology, and Speciation

Modern “omics” studies (genomics, transcriptomics etc.) have been used to great effect to understand the evolutionary histories of dozens of species. Representative species genomes from the elasmobranchs, however, have only recently been sequenced due to the difficulties associated with their very large genomes. The genome sequence for cartilaginous fish has historically been represented by the much smaller genome of the elephant fish (*Callorhinchus milli*), a chimera (a fellow member of class Chondrichthyes but of subclass Holocephali, not Elasmobranchii). Hara, et al. [16] assembled de-novo genome sequences for the brownbanded bamboo shark (*Chiloscyllium punctatum*), the cloudy catshark (*Scyliorhinus torazame*), and the whale shark (*Rhincodon typus*) and compared these genomes to known genomes, such as those of humans and other vertebrates. Hara, et al. found that elasmobranchs boasted large, slowly-evolving genomes rife with non-coding sections (underscoring the need to create genome maps for elasmobranchs, rather than relying on those of a related group), and shared many gene regions with humans, regulating processes such as homeostasis, body plan, regulation. Such findings indicate that these genes were present in the genomes of common ancestors earlier in the tree of life than previously thought, all the way to a hypothesized “common ancestor of jawed vertebrates.” Finally, Hara, et al. found that olfactory (smell-related) and opsin (sight-related) receptor family genes were relatively scant, indicating unique sensory adaptations to evaluate the different niches they occupy. The opsin gene repertoires, for example, differed between the deep-diving whale sharks and cloudy catsharks, the former having pigments that absorbed light of a longer wavelength, indicating adaptations to deeper, darker habitats.

The timing of speciation events by genetic analyses combined with a molecular clock can also be used to generate hypotheses about past speciation events. For example, Corrigan and Beheregaray’s [10] hypothesized radiation within Orectolobidae was due to new barriers to dispersal arising due to shrinking sea levels, caused by the rapid formation of glaciers, in a period of global climate cooling. These barriers included land bridges and temperature gradients. The loss of habitat in some places led to prezygotic isolation between individuals, but also to suitable habitats appearing elsewhere; the ancestors of early wobbegong simply specialized to these new habitats. Similarly, Quattro, et al. (2006) [28] infers that the divergence between the Atlantic and Indo-Pacific populations of scalloped hammerhead was the result of the sudden uplift of the Isthmus of Panama, although he could not propose a specific mechanism. Sorenson, et al. [33] compared estimated divergence time of elasmobranch species, derived from the genetic analysis of 268 different species of shark, with habitat preference of the species collected to discern evolutionary changes as they related to environmental changes. Sorenson, et al. found that the Squalimorph and Galeomorph lineages diverged from the most recent common ancestor of all sharks and diverged into lineages occupying deep sea and shelf habitats, respectively. From the Galeomorphs, the Lamniformes evolved to take up more pelagic niches, while the rest of the Galeomorphs evolved to fill more littoral niches. Curiously, the study was unable to support the commonly-held hypothesis that higher diversification rates occurred in the coastal or shelf areas, as opposed to the deep ocean areas; that is, the Squalimorphs of the deep ocean had speciated more than the pelagic and coastal Galeomorphs (except for coral reef-favoring lineages of Carcharhinids). Sorenson, et al. suggests that this apparent relationship between habitat and diversification rates was simply a product of time; the Squalimorphs found early success in deep water and were able to radiate sooner. Indeed, Sorenson, et al. concludes that rates of speciation are not so much dependent on habitat productivity (although the high productivity and specialization opportunity of coral reefs will drive future speciation) but on the ability to
colonize and specialize in new habitats and to survive drastic changes to existing habitats.

**Future Challenges to Conservation**

Genetic and morphological analyses are crucial to the study and conservation of elasmobranchs, which is both imperative and time-sensitive. Elasmobranchs are long-lived but reach sexual maturity slowly and have few offspring, meaning that lost individuals cannot easily be replaced. Additionally, elasmobranchs are often found at the top of the food webs of the marine ecosystems in which they occur, creating a disproportionate influence per individual on their surrounding communities. Their niche within their ecosystems can generally be described as specialized predators of bony fish, crustaceans, and other organisms, thereby regulating populations of these lower trophic level organisms [9]. Their immense biodiversity allows elasmobranchs to fill many niches within marine ecosystems and, at times, highly-specialized niches within very limited geographic ranges [14]. This biodiversity facilitates ecosystem services at the coasts and in open oceans, and its destruction threatens the viability of these services and the societies that rely on them [13].

Although speciation is not typically something that occurs within a normal lifetime or beyond, it can be affected by anthropogenic actions. Simply put, humans have an inordinate ability to modify their environment. As Sorenson, et al. [33] puts it, “biodiversity dynamics at the macroevolutionary scale are strongly influenced by the environment” (p. 1536). The extraordinary ability of humans to modify the environment will have repercussions on the evolutionary trajectory of elasmobranch species. Modifications can take the form of rising sea levels, sedimentation, and new gradients in temperature or water chemistry, all of which can alter or restrict movement of populations across space, potentially leading to isolation [13,14]. Of course, these modifications with the introduction of pollutants such as microplastics can affect elasmobranchs in the short term, physiologically and ecologically [26]. Much like in Australia and Panama, the changing environment will not only prezygotically isolate existing individuals, but will also select for species and traits that will tolerate these changes and alter or create niches to be filled. Such changes will ultimately drive speciation that will inevitably affect ecosystems and fisheries [10,25,28].

Coastal fisheries, more so than less visible anthropomorphic changes to the ecosystem, present a more dire threat to elasmobranch species. Elasmobranchs are under immense, likely unsustainable fishing pressure around the world, threatening both known and cryptic species alike [5]. Unfortunately, biodiversity studies are lacking in many coastal areas, leading to incomplete data being used in fisheries management assessments, especially with the occurrence of cryptic species [14,18]. Current fishing regulations could allow for the continued harvest of cryptic species under the guidelines set for its previous species identification, without knowing anything about its ecology or vulnerability to fishing pressures. Without even knowing of their existence, cryptic species could go extinct, potentially leaving niches unfulfilled and reducing genetic diversity, to the detriment of the ecosystem and the fishery [14,17,24,28]. Indeed, current phylogenetic studies indicate that understandings about evolutionary relationships between species that we know about and total biodiversity are still incomplete and will require further study to properly determine [3,9,18,35]. Gaps in knowledge and understanding stand to threaten the survivability of many species of elasmobranch, including those unknown to science. A loss in biodiversity of coastal elasmobranchs threatens to destabilize already-imperiled coastal ecosystems. Not only would coastal fishing industries stand to lose potential resources, but such a destabilization threatens the many other organisms and ecosystem services that fishermen, mainly artisanal, depend on for life and livelihood [13,18].

Further study in evolutionary history may seem less productive than simple ecological study in preserving elasmobranchs and other species, but as Hendry, et al. [18] argues, the study of evolutionary history would complement and enhance current ecological study and policy. Not only would further study likely unearth new biodiversity and predict cryptic species, through a combination of morphological or phylogenetic
studies, but it would also allow for study into possible environmental drivers of such evolution, such as shrinking sea levels and its effects on Orectolobiformes found by Corrigan and Beheregaray [10]. Hypotheses about the effect of past conditions on extinct and extant species could allow for evaluation on the possible effects of modern disturbances on elasmobranch species, especially those caused or influenced by human activity, as well as how they might affect the surrounding ecosystem. Furthermore, study into phylogeny may highlight species and populations with genetic diversity, evolutionary uniqueness, and functional diversity (the range of niches performed by organisms within an environment) within elasmobranchs, allowing conservationists to target conservation choices with likely-limited resources [3]. Context is key to understanding the biodiversity in elasmobranchs we see today. Understanding the evolutionary context behind elasmobranch speciation would allow conservationists to better anticipate and address complications to species’ survivability brought about by anthropogenic-driven environmental change.

Conclusion

Today, traditional studies of fossil and specimen morphology have combined with cutting-edge phylogenetic and genomic analyses to create a more accurate view of how current elasmobranch species evolved. Examinations of ancient lineages give us the context behind the morphology, genetics, and past speciation of today’s known species. Simultaneously, phylogenetic and computer-aided morphological analyses allow for the calibration of these lineages and the prediction of the presence of cryptic species. The Elasmobranch class is more than what is seen in our media, pop culture, and aquariums. Current marine conservation policy risks excluding cryptic and lesser-known species and ignoring evolutionary trajectory, to the detriment of the whole ecosystem. To neglect the evolutionary history of cryptic species, many of which may perform crucial ecosystem services, risks ruining ecosystems and fisheries, negatively altering fishermen’s livelihoods and compromising conservation efforts globally. Ultimately, phylogenetic studies and morphological studies should be continued to best determine the presence of new or range-expanded species, as well as areas of genetic and ecological importance. The results of these studies should be used to improve existing evolutionary theory, which in turn should be used to identify trends in speciation. Finally, these studies should be used to inform sound conservation practices that ensure the best chances for the survival of species of elasmobranchs and other marine life for generations to come.

References


The Relationship between Hospital Patient Safety Culture and Patient Satisfaction: A Systematic Literature Review

Joel Bass¹, Christian Fields², Lauryn Vaughn², Amy Ratley², Fallon Davis²

Faculty Mentor: Katherine Meese, PhD, MPH

¹Department of Health Services Administration, The University of Alabama at Birmingham, Birmingham, AL 35205
²Department of Clinical & Diagnostic Sciences, The University of Alabama at Birmingham, Birmingham, AL 35205

Both patient satisfaction and patient safety have become important measures of success for hospitals in the United States. However, research findings have varied about whether and how these constructs may be related. The purpose of this research project was to conduct a systematic literature review related to the research question, “What is the relationship between hospitals’ patient safety cultures and patient satisfactions?” We first determined keywords, which included 16 variations of the terms safety culture, patient experience, and hospital. The keywords were searched in databases including PubMed, CINAHL, and ABI/Inform within the years 2004 to 2020. The initial search identified 26,287 articles. Using the PRISMA framework, we applied elimination criteria which included non-peer reviewed, non-English, non-U.S.-based, unrelated, and duplicate articles. After applying this criteria, nine articles were selected for full-text review. While examining the nine articles, there were seven articles that found a positive relationship between patient safety culture and patient satisfaction compared to the two articles that found no statistically significant relationship. Although all of the articles explored the relationship between patient safety culture and patient satisfaction, each of them had a unique study design (systematic literature review, cross-sectional, retrospective, observational, etc.) and utilized different measurement instruments, which led to varying findings. This systematic review is the first step in understanding the degree of empirical support for the relationship between patient safety culture and patient experience. Overall, there is evidence to suggest that there is a positive relationship between these two important hospital objectives. This evidence has the potential to influence our perception of patient safety culture and its effects; however, there is still a need for more specific studies on this topic to fully understand this nuanced relationship. This study found that patient safety culture and patient satisfaction are two very important factors on their own, but the relationship between them is still being uncovered.

Introduction

In the last 16 years, there has been an increase in the use of the Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) Survey, which measures patient satisfaction. This has expanded the amount of data available for research on this important element of patient care. In recent years, there has been a rise in the number of review articles that examine patient safety culture or patient satisfaction, but there have only been a few published articles that examine the relationship between the two. The last article that investigated the correlation between patient safety culture and patient satisfaction was published around 2017, using 2012 data. Patient safety culture is defined as “the values shared among organization members about what is important, their beliefs about how things operate in the organization, and the interaction of these with work units and organizational structures and systems, which together produce behavioral norms in the organization that promote safety” [6]. The definition that we used for patient satisfaction is “a commonly used, critical indicator in evaluation of health care service quality as patients have contributor, target, and reformer roles in quality assurance” [4].

It follows that patient safety culture will have a correlation with patient satisfaction. It
seems as though patients who are in a safer environment will feel safer and therefore be more satisfied. The safety culture regarding patients should also affect hospital staff and the various actions they take with patients. These actions taken collectively will impact the experience a patient has and thus how satisfied patients are with their care. However, when examining previous research on the relationships between safety culture and patient satisfaction, it is evident that the significance of this relationship has not been definitively established. The purpose of this study is to further explore the relationship between patient safety culture and patient experience through a systematic review of articles examining this relationship within U.S. hospitals.

Methods

This systematic literature review was created using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) model. The date range chosen for reporting was 2004 to the present, as 2004 was the year HCAHPS patient satisfaction surveys were standardized within the United States. The databases searched included Pubmed, CINAHL, and ABI/Inform. There were a total of 17 search terms, each a variation of the terms hospital, patient satisfaction, and patient safety culture.

The initial search reported 23,495 articles. These were then uploaded into Covidence, a systematic review management system. Duplicates, non-English, non-peer reviewed, specialty-related, and non-hospital settings comprised the exclusion criteria. 5,886 duplicates were removed, leaving 17,609 articles for initial screening of the titles for relevance.

Articles were included if they met the following criteria:
1. Published in English and in the United States
2. Peer-reviewed
3. Discussed a direct relationship between patient safety culture and patient satisfaction
4. Non-specialty related areas
5. Included a hospital setting

From the 17,609 articles, a team of five student researchers screened these articles by title, eliminating those that did not meet the criteria above. The remaining 1,223 studies were screened by title and abstract (Figure 1). A total of nine studies were included for full-text review. The bibliographies of these articles were hand-searched to identify any additional articles for inclusion. These studies were then transferred into EndNote, a citation management software. From the nine included articles, the following information was pulled from the articles: authors, title of article, year of publication, abstract, study design, method type, sample size, type of organization, main relationships tested, and statistically significant findings (Table 1).
FIGURE 1
PRISMA Search Strategy

Articles identified through database searches (n=23,495)

Duplicates removed (n=5,386)

Articles screened by title and abstract (n=17,609)

Articles excluded (n=16,386)

Full-text articles assessed for eligibility (n=1,223)

Full-text articles excluded with reasons* (n=1,214)

Articles included in final review (n=9)

*Legend

<table>
<thead>
<tr>
<th>Reasons for exclusion</th>
<th>Number of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Not related</td>
<td>330</td>
</tr>
<tr>
<td>2 Did not include patient safety</td>
<td>308</td>
</tr>
<tr>
<td>3 Did not include patient satisfaction/experience</td>
<td>241</td>
</tr>
<tr>
<td>4 Wrong study design</td>
<td>169</td>
</tr>
<tr>
<td>5 Not U.S.</td>
<td>146</td>
</tr>
<tr>
<td>6 Wrong setting</td>
<td>15</td>
</tr>
<tr>
<td>7 Duplicate</td>
<td>5</td>
</tr>
</tbody>
</table>
Overall, the nine articles that we examined had mixed results regarding the relationship between patient safety culture and patient satisfaction. Within the articles, there were three cross-sectional studies, four systematic reviews, one retrospective cohort study, and one retrospective observational study. Of these, there were seven statistically significant positive relationships and two that failed to reach significance; however, each article discussed various aspects related to patient satisfaction and safety culture. No two articles examined the same exact set of information. An example of this is the varying sets of data and sample sizes. For instance, each article had vastly different amounts of hospitals being examined such as Abrahamson et al., 2016 with 45 hospitals, compared to Isaac et al., 2010 with 927 hospitals.

Data Sources
Out of the nine articles, four used the study design of systematic reviews, three were cross-sectional study designs, one was a retrospective observational study, and one was a retrospective cohort study. All of the articles used hospitals in the United States as the study setting. Sample size varied among the articles. The articles had sample sizes in the units of studies, articles, hospitals, and inpatients. The articles by Anhang et al., 2014, Tevis et al., 2014, and Mazurenko et al., 2017 have a sample size of 31 studies, 10 studies, and 41 articles, respectively. The articles by Abrahamson et al., 2016, Sorra et al., 2012, McClelland et al., 2014, Stein et al., 2015, and Isaac et al., 2010 have sample sizes of 45 hospitals, 73 hospitals, 269 hospitals, 4,605 hospitals, and 927 hospitals, respectively. The article by DeVos et al., 2019 is the only article that has a sample size of inpatients, at 4,236. All nine of the articles used HCAHPS as a survey tool, but other survey tools were also used. In addition to HCAHPS, Abrahamson et al., 2016 used the Agency for Healthcare Research and Quality (AHRQ) survey. McClelland et al., 2014 utilized the likelihood of recommendation and surveys collected from hospital executives. Additionally, Stein et al., 2015 used hospital acquired condition (HAC) and patient safety indicators (PSI) data from the Centers for Medicare and Medicaid Services (CMS); Tevis et al., 2014 also used data from CMS. DeVos et al., 2019 utilized the Picker Patient Experience Questionnaire, and Isaac et al., 2010 used data from the Hospital Quality Alliance.
Table 1
Characteristics of the selected studies in the systematic review

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Title</th>
<th>Study design/ Sample size</th>
<th>Study results and significant conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamson et al., 2016</td>
<td>The Relationship Between Nurse-Reported Safety Culture and the Patient Experience</td>
<td>Cross-sectional study (N=45 hospitals)</td>
<td>Using data from a multistate sample of hospitals, the relationship was tested by multivariate mixed-effects regression models. Within the variables of the HCAHPS and AHRQ surveys, the relationship was significantly correlated within multiple domains, specifically adequate staffing (p&lt;0.05).</td>
</tr>
<tr>
<td>Anhang et al., 2014</td>
<td>Examining the role of patient experience surveys in measuring health care quality</td>
<td>Systematic review (N=34 studies)</td>
<td>Better patient care experiences were associated with better patient safety in hospitals. No statistical analysis was performed.</td>
</tr>
<tr>
<td>DeVos et al., 2019</td>
<td>The Association Between Complications, Incidents, and Patient Experience: Retrospective Linkage of Routine Patient Experience Surveys and Safety Data</td>
<td>Retrospective observational study (N=4,236 inpatients)</td>
<td>There is little to no evidence to support a correlation between patient satisfaction and patient safety culture. In bivariate analyses, no association was found between complications/incidents and overall patient experience (p=0.09).</td>
</tr>
<tr>
<td>Isaac et al., 2010</td>
<td>The relationship between patients’ perception of care and measures of hospital quality and safety</td>
<td>Retrospective cohort study (N=927 hospitals)</td>
<td>A strong positive association between the two measures was found. The correlation coefficients ranged from 0.15 to 0.63 and p&lt;0.05 for all.</td>
</tr>
<tr>
<td>Mazurenko et al., 2017</td>
<td>Predictors of Hospital Patient Satisfaction as Measured by HCAHPS: A Systematic Review</td>
<td>Systematic review (N=41 articles)</td>
<td>This study showed that hospital patient safety culture is associated with a higher rate of patient satisfaction. Most of the studies examined patient satisfaction compared to various hospital-level predictors. The predictors were consistently shown to have positive and negative relationships with positive relationships prevailing.</td>
</tr>
<tr>
<td>McClelland et al., 2014</td>
<td>Compassion Practices and HCAHPS: Does Rewarding and</td>
<td>Cross-sectional study</td>
<td>Positive associations were identified between specific organizational practices and satisfaction. Also, there was a positive</td>
</tr>
</tbody>
</table>
Supporting Workplace Compassion Influence Patient Perceptions? (N=269 hospitals) association between the likelihood of recommendation and hospital rating with compassion practices. Overall, p<0.05 for hospital rating and likelihood of recommendation.

Sorra et al., 2012 Exploring relationships between patient safety culture and patients’ assessments of hospital care Cross-sectional study (N=73 hospitals) Positive perceptions of patient safety culture strongly correlated to patient satisfaction (r = 0.41, p< 0.01).

Stein et al., 2015 Patients’ Perceptions of Care Are Associated With Quality of Hospital Care: A Survey of 4,605 Hospitals Systematic review (N=4,605 hospitals) There is an inverse relationship between patient experience and complication rates. Generally, the results suggest that patient experience is positively correlated with safety culture. There is strong statistical discussion about how different hospital acquired conditions and patient safety indicators affect HCAHPS response within Table 3.

Tevis et al., 2014 Can patients reliably identify safe, high quality care? Systematic review (N=10 studies) There are differing associations between patient safety culture and patient satisfaction. No statistical tests were performed.

**Association Between Patient Satisfaction and Patient Safety Culture**

When discussing the articles, they can be grouped together based on similar findings. There were two articles that found no relationship between patient safety culture and patient satisfaction [5,12]. DeVos et al., 2019 used a multivariable analysis, while Tevis et al., 2014 was a systematic review and did not perform statistical tests. DeVos et al., 2019 used patient-reported problems from the Picker Patient Experience Questionnaire to find a relationship. They found that if patients reported problems, they were more likely to have a non-positive experience; however, they also found that continuity of problems increased the chance for a non-positive experience. The article then went on to say that this was a good way to find ways to make care better, but there was still no correlation between complications or incidents and overall patient experience (p=0.09). Tevis et al., 2014 was a systematic literature review and examined various articles related to safety culture and satisfaction and the HCAHPS survey. Out of ten articles, they found varying results in each one and thus concluded that there was no definitive relationship.

Other articles found a positive relationship between patient safety culture and patient satisfaction [1,2,7,8,9,10,11]. Three of these were systematic reviews and did not have any statistical tests; rather, they compiled information from other articles [2,8,11]. In their review, Anhang et al., 2014 specifically studied the association between patient experiences and other measures of healthcare quality. There was a positive association between positive care experience and patient adherence and influence of adherence on clinical outcomes. There was also support found for an association between positive patient experience and best practice clinical processes, better hospital safety culture, and lower unnecessary utilization [2,7]. These findings suggest that patient experience is an appropriate measure for quality of care. The article written by Mazurenko et al., 2017.
discussed numerous predictors that were associated with patient satisfaction. While this systematic review did not directly discuss the relationship between patient safety culture and patient satisfaction, it did describe indicators that have an impact on patient satisfaction, such as safety culture. Stein et al., 2015 indicated an inverse relationship between complication rates in hospitals and patient experience. In addition to these systematic reviews that found a positive correlation between patient safety culture and patient satisfaction, there was an article that examined the relationship between the Hospital Survey on Patient Safety Culture (HSOPS) and the HCAHPS survey using a multiple regression analysis [10]. Sorra et al., 2012 found positive perceptions of patient safety culture had a strong correlation to patient satisfaction (r=0.41, p<0.01). Isaac et al., 2010 alternatively used a bivariate analysis and found that there was a strong positive association between patients’ perception of care and measures of hospital quality and safety. The correlation coefficients ranged from 0.15 to 0.63, and overall p<0.05. Another article examined the relationship between patient experience and nurse-reported safety culture [1]. Abrahamson et al., 2016 used a multivariate mixed-effects regression model. In their analysis, they used data from the HCAHPS and Agency for Health Research and Quality (AHRQ) surveys. There was a high correlation among the category of adequate staffing (p<0.05), which yielded a positive relationship between patient experience and teamwork, sufficient staffing, and organizational learning [1]. McClelland et al., 2014 used a regression analysis and found positive associations between specific organizational practices and satisfaction, as well as between likelihood of recommendation and hospital rating with compassion practices. There was a p<0.05 for hospital rating and likelihood of recommendation.

Organizational Factors

Numerous organizational factors were found to play a role in the relationship between patient safety culture and patient satisfaction. Some organizational factors that played a role included bed size, teaching status, safety net hospital status, and for-profit/non-profit status. Overall, safety net, larger (>100 beds), and for-profit hospitals were associated with lower patient satisfaction [8]. Teaching status was also determined to be a noteworthy factor in this relationship. Some studies found that teaching status was associated with patient satisfaction, while other studies failed to examine this relationship. A potential explanation of this relationship could be the fact that a patient’s perception of teaching hospitals has something to do with increased access to new technology and research. There is a limited amount of evidence that suggests patients tend to equate the level of technological sophistication with better care [3]. Also, within hospitals, the use of trainees such as residents and fellows may contribute to a lower overall satisfaction score because of the increased amount of clinical staff in the patient’s presence. Of these factors noted, some demonstrate a positive role in the relationship, while some demonstrate a negative role. A further evaluation of organizational factors that affect the relationship between
patient safety culture and patient satisfaction is needed. Of the predictors that affected patient satisfaction, researchers [3] found that certain work environment attributes such as an organizational culture that emphasizes compassion, the presence of well-defined nursing standards for high-quality care, a greater cultural competency, and a more positive staff perception of a safety culture were positively associated with patient satisfaction [3].

Patient or Provider-level Factors

Out of the nine articles reviewed, there were various patient and provider-level factors within seven articles. Two had neither patient nor provider-level factors. Abrahamson et al., 2016 examined the relationship between nurse-reported safety culture and the patient experience. This article examined many safety culture domains; however, teamwork, adequate staffing, and organizational learning were more prominent than other factors on the achievement of a positive patient experience. Teamwork was a provider-level factor that was also present in Sorra et al., 2012. Patient-level factors this study examined were the education level of patients and the likelihood to recommend the hospital. It was also noted that many studies examined the level of patient-provider communication and how it contributed to the various factors at play [2,10,11]. It was found that there were better-reported patient experiences when physicians considered the patient to be a “whole person” [2]. DeVos et al., 2019 examined how hospital-related incidents affected the patient experience. The findings concluded that the incidents that happen to patients have very little effect on how satisfied they are in the end. Two of the articles assessed patient satisfaction compared to patient safety indicators (PSIs). PSIs were developed by the Agency for Healthcare Research and Quality (AHRQ) to determine various characteristics such as demographics or length of stay [7]. Studies use these PSIs to find a relationship between patient safety culture and experience. Isaac et al., 2010 specifically compared lower decubitus ulcer rates to find that these resulted in better patient experience; however, one article found that while using HCAHPS data and PSIs there were conflicting results [12].

Discussion

Given the conflicting findings in prior research, additional research is needed to clarify the relationship between patient safety culture and patient satisfaction. As stated previously, the articles we examined had many conflicting results and limitations. The sample sizes were not sufficient enough to gather appropriate data to fully represent the population as a whole [1,7,10]. In addition, PSIs rely on accurate coding within billing data, and their validity as safety measures has not been well established [7]. Also, some articles worthy of inclusion may have been missed, as there have not been many published articles related to this topic [8]. Study designs can also be designated as limitations in a number of articles we reviewed [9,11]. Hospital-acquired conditions (HACs) primarily dealt with Medicare beneficiaries and were not corrected for demographics and differences, while HCAHPS was a combination of both Medicare and non-Medicare beneficiaries [11]. The response rate and fulfillment of patient expectations were also limitations [5]. In addition, some articles did not explicitly use the terms patient satisfaction or patient safety culture. The article by McClelland et al., 2014 used the term “compassion practices” to discuss these various aspects. The general idea was the same for these various articles within their discussions.

In this review, multiple studies were analyzed to examine the relationship between patient satisfaction and patient safety culture. Previously, there have been studies done on each component individually, but few have been conducted on both components. This review showed that there were positive and non-significant relationships. Further research is needed to truly understand the relationship and the potential these components have.

References

Medical Cannabis in the Treatment of Parkinson’s Disease

Colin Begley¹,², Alexandra Begley², Łukasz Ciesla¹

Faculty Mentor: Łukasz Ciesla¹

¹Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487
²DENT Neurologic Institute, Buffalo, NY 14226

Preclinical research has suggested medical cannabis (MC), a neuroprotective and anti-inflammatory substance, to be therapeutic in the treatment of neurodegenerative diseases such as Parkinson’s disease (PD). The role of the endocannabinoid system in modulating dopaminergic neurotransmission, in addition to other neurotransmitter systems, in the basal ganglia is central to this notion. Though current PD medications are usually efficacious in reducing motor symptoms, they do not alleviate non-motor symptoms or impact disease progression. Furthermore, these drugs can have numerous adverse effects. Largely due to federal Schedule I regulations on marijuana, there is a lack of large-sample randomized placebo-controlled trials that have been conducted on MC in PD. This retrospective study on MC efficacy in 62 PD patients found improvement of 77% of the sample in PD motor symptoms (spasticity, tremor, rigidity, bradykinesia, and dyskinesia) and of 50% in non-motor PD symptoms (sleep disturbance, anxiety, depressed mood, and nausea). On MC, 85% of patients with pain experienced pain reduction, whereas 54% of patients on opioids either discontinued or decreased their use by more than half. MC was well tolerated in this population with side effects of relatively low severity. Because most patients were also taking traditional PD medications with MC therapy, the results propose MC to be an effective add-on therapy to traditional PD treatment regimens to help relieve refractory symptoms, treat non-motor symptoms, and/or alleviate side effects of PD medications such as levodopa-induced dyskinesia.

Introduction

Although marijuana has been categorized as a Schedule I federally-regulated controlled substance since 1970, 33 U.S. states and the District of Columbia have active legislation allowing the prescription of medical cannabis (MC). Parkinson’s disease (PD) is an indication for MC in 12 of these states; however, PD patients can be approved for MC in other states through state-approved associated symptoms such as pain and muscle spasm, and both Oklahoma and the District of Columbia allow MC prescription for any physician-recommended condition (Figure 1, Figure 2) [1,2].

![Figure 1: Mapping Medical Cannabis: U.S. Comprehensive MC-Program States [1,2]](image-url)
In the U.S., PD is the second-most common neurodegenerative disorder and the 14th leading cause of death across all age groups. PD is a movement disorder characterized by the destruction of dopamine-producing neurons in the movement-coordinating region of the basal ganglia, which causes dopamine deficiency and subsequent motor disability [3]. The four hallmark motor symptoms of PD are resting tremor, bradykinesia, muscular rigidity, and postural instability. In addition, muscle spasms can also be observed in PD patients. As PD also impacts the serotonergic, cholinergic, noradrenergic, and autonomic nervous systems, non-motor symptoms are observed in an estimated 90% of PD patients, including sleep disturbance, depression, anxiety, memory loss, and psychosis [4].

**Background**

**The Endocannabinoid System**

It is thought that the endocannabinoid (ECB) system, a complex system involved in movement coordination, pain regulation, pleasure, appetite, and memory, plays a role in modulating the dopaminergic system in addition to the GABAergic, glutamatergic, cholinergic, noradrenergic, and autonomic nervous systems. Non-motor symptoms are observed in an estimated 90% of PD patients, including sleep disturbance, depression, anxiety, memory loss, and psychosis [4].

**The ECB System and Parkinson’s Disease**

The highest densities of CB1 receptors and AEA are localized in the major movement-controlling regions of the basal ganglia: the substantia nigra and globus pallidus [3]. In these regions, CB1 receptors are located on GABAergic and glutamatergic neurons, while CB2s are located on dopaminergic neurons. ECB system activation induces decreased dopaminergic activity and subsequent motor inhibition. As such, the ECB system in PD patients deviates from that of healthy individuals. It has been shown that people with PD have a heightened level of CB1 receptor expression and increased ECB activity in their basal ganglia. Additionally, increased levels of ECBs such as AEA have been discovered in untreated patients’ cerebrospinal fluid [5]. While it may seem logical to presume that exogenous cannabinoids are ineffective in PD treatment, studies exploring both agonism and antagonism of the ECB system have revealed efficacy in treating PD symptoms [6].

Notably, cannabinoids have neuroprotective properties that slow neurodegeneration by reducing both glutamatergic pathway excitotoxicity and oxidative damage [7]. Therefore, cannabinoids are likely valuable in the treatment of neurodegenerative diseases such as Parkinson’s because this neurodegenerative process occurs through the exact excitotoxic and oxidative...
processes that cannabinoids counteract. The cannabinoids also exhibit anti-inflammatory properties, namely through CB2 receptor activation on microglia that decreases the prevalence of pro-inflammatory cytokines and increases that of anti-inflammatory cytokines. Since neuroinflammation mediated through microglia in the substantia nigra has been found to exacerbate PD progression and dopamine neuron loss, cannabinoids are also of value in PD treatment for their anti-inflammatory properties [8].

**Medical Cannabis**

The 2 major components of MC are Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD). THC, similar in composition to AEA, is a psychoactive substance that is a partial agonist at both CB1 and CB2 receptors. In comparison, CBD is a non-psychoactive, neuroprotective, and anti-inflammatory substance that exhibits indirect antagonism of cannabinoid agonists, which is thought to help potentiate THC’s effects by increasing availability of CB1 [3]. CBD can also decrease both reuptake and degradation of AEA, which in turn has the potential to increase CB1 activation [8]. Finally, CBD is a negative allosteric modulator of CB1, which allows CBD to balance out the psychoactive, euphoric effects of THC [10]. Medical marijuana dispensaries produce MC in several different THC:CBD ratios to provide optimal therapeutic effects for patients based on each component’s pharmacotherapeutic properties [1].

**The Problem**

Currently, all traditional PD medications target some aspect of dopamine neurotransmission, and while these medications are often effective in treating PD motor symptoms, they do not halt disease progression and do not treat non-motor PD symptoms such as pain, sleep disturbance, anxiety, and depression [3]. Levodopa combinations are considered the gold standard for PD treatment and are therefore commonly prescribed first. Though this medication tends to cause near-complete symptom eradication for the first couple years of treatment (the “honeymoon” period), after this time, patients often experience motor fluctuations consisting of “off” periods of immobility [12]. Then, patients must continually increase their levodopa dosing frequency or add other medications to avoid motor fluctuation [11,12].

These drugs also can have numerous adverse effects; levodopa can bring on levodopa-induced dyskinesia, and levodopa or other medications can precipitate numerous side effects including nausea, dizziness, headache, orthostatic hypotension, nightmares, and hallucinations (Figure 3) [11]. Sometimes side effects occur in such severity that patients cannot tolerate the therapy, while other patients may find their PD symptoms refractory to any of these treatment options [13]. For these reasons, there is an obvious need for new targets of PD treatment that mitigate adverse effects, treat non-motor PD symptoms, and delay disease progression. Given the interaction of the endocannabinoid system with the dopamine, GABA, glutamate, serotonin, and acetylcholine neurotransmission pathways and its positive effects on neurodegeneration and neuroinflammation, MC is a promising avenue for a novel, non-dopamine-targeted treatment of PD [3].
Table: Traditional Parkinson’s Medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>Mechanism of Action</th>
<th>Usage</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine Precursor:</td>
<td>Provision of dopamine precursors to replenish dopamine</td>
<td>First-line treatment of PD motor</td>
<td>Dyskinesia, motor fluctuation, dizziness,</td>
</tr>
<tr>
<td>Carbidopa-Levodopa</td>
<td>deficit</td>
<td>symptoms</td>
<td>orthostatic hypotension, depression, headache</td>
</tr>
<tr>
<td>combinations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine Agonist:</td>
<td>Direct stimulation of postsynaptic dopamine receptors</td>
<td>Monotherapy, or in addition to</td>
<td>Nightmares, hallucinations, insomnia, nausea,</td>
</tr>
<tr>
<td>Pramipexole, Ropinirole</td>
<td></td>
<td>levodopa for increased symptom</td>
<td>dizziness, orthostatic hypotension, precipitation of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>impulse-control disorder (i.e. gambling,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>hypersexuality)</td>
</tr>
<tr>
<td>Monoamine Oxidase B</td>
<td>Inhibition of dopamine catabolism</td>
<td>Monotherapy, or in addition to</td>
<td>Nausea, dizziness, orthostatic hypotension, headache,</td>
</tr>
<tr>
<td>B Inhibitors:</td>
<td></td>
<td>levodopa for increased symptom</td>
<td>insomnia</td>
</tr>
<tr>
<td>Selegiline, Rasagiline</td>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>COMT Inhibitors:</td>
<td>Prevents degradation of levodopa to increase its</td>
<td>Add-on to levodopa therapy</td>
<td>Nausea, vomiting, diarrhea, urine discoloration,</td>
</tr>
<tr>
<td>Entacapone, Tolcapone</td>
<td>availability in the basal ganglia</td>
<td></td>
<td>dizziness, confusion, worsening of LID</td>
</tr>
</tbody>
</table>

Figure 3: Traditional Parkinson’s Medications [11]

Though randomized placebo-controlled trials are ideal to investigate this topic, the federal categorization of marijuana as a Schedule I Controlled Substance has rendered carrying out these studies exceedingly difficult, as only institutions with a Schedule I license may conduct them [6]. Because of these regulations, a retrospective chart analysis of Parkinson’s patients on MC therapy was conducted to investigate MC efficacy in both motor and non-motor symptom management in addition to side effect profiles and dosing information. This study design carries the following limitations: patients were taking several different medications during the study that have an unknown effect on MC therapy, and the results rely on information that the provider documented in the patient chart. Additionally, no information on long-term effects was available.

Literature Review

Given the aforementioned Schedule I federal regulations on marijuana, currently available studies on MC use in PD were conducted in a country other than the U.S., used Schedule II synthetic cannabinoids, or were simply based on patient surveys. The studies also have small sample sizes and conflicting data. Therefore, results are neither conclusive nor replicable.

An open-label observational study conducted at a tertiary-care center in Israel evaluated the response of 22 PD patients who were on MC for at least two months and smoking 0.5 grams of cannabis. At 30 minutes post-cannabis administration, the study found significant improvement of patients’ mean score on the Unified Parkinson Disease Rating Scale (UPDRS) by almost ten points. Within the UPDRS analysis, significant improvement was
found in patients’ tremors, rigidity, and to a lesser extent, bradykinesia. This study also reported significant improvement in patients’ pain based on the Present Pain Intensity (PPI) and Visual Analog Scale (VAS), and in patient-reported sleep improvement [13].

The same facility in Israel conducted a similar open-label observational study on 20 PD patients approved for MC therapy, two of which ingested MC via vaporization and the rest of which through smoking. This study also found significant improvement in UPDRS scores 30 minutes post-cannabis administration by about eight points as evaluated by one blinded and one non-blinded rater, and significant improvement in the Pain Rating Index (PRI) and VAS. This research also evaluated pain response objectively through changes in thermal quantitative sensory testing (QST), which was conducted before cannabis administration, 30 minutes after cannabis administration, and at a follow-up evaluation after a median of 14 weeks of MC therapy. This portion of the study found mean cold pain threshold to significantly decrease in those patients who smoked MC at 30 minutes post-cannabis administration and heat pain threshold to significantly decrease in all patients at the follow-up evaluation, which provides additional evidence for the role of MC in pain modulation [14].

A survey sent out to all PD patients of the Prague Movement Disorder Center found that, of 85 patients who reported cannabis use, 46% reported efficacy in treatment of PD symptoms. More specifically, reported improvements were 45% for bradykinesia, 38% for rigidity, 31% for tremor, and 14% for levodopa-induced dyskinesia [15].

Another survey available via the Michael J. Fox Foundation and the National Multiple Sclerosis Society websites sampled both PD and multiple sclerosis patients on cannabis use and efficacy. The results found that the 260 cannabis-user respondents reported an average cannabis efficacy of 6.4 on the Likert scale in symptom improvement, and that 59% of users reported a decrease in use of other medications with cannabis. Patients who used cannabis reported a lower disability level, as they scored higher on Guy’s Neurological Status Scale (GNDS) than the group of 335 respondents who did not use cannabis. Specifically, improvement was noted in the mood, memory, and fatigue domains of those using cannabis [16].

One randomized, double-blind, placebo-controlled trial studied the response of 21 PD patients to CBD capsules through improvement rated by the UPDRS and Parkinson’s Disease Questionnaire-39 (PDQ-39) after six weeks of therapy. There were three groups in the study: a placebo group and two experimental groups, with one taking 75 mg/day of CBD and the other taking 300 mg/day. While there were no statistically significant differences found among the groups in the UPDRS, there was a statistically significant difference between the placebo group and the 300 mg/day CBD group in the PDQ-39. These results suggested CBD to have a positive effect on quality of life in PD patients [17].

Finally, two randomized, double-blind, placebo-controlled crossover trials evaluated the effect of cannabinoid therapies on levodopa-induced dyskinesia (LID). The first study was conducted on five PD patients with stable LID for at least 25 to 50 percent of the day and assessed patient response to nabilone, a synthetic CB1 and CB2 agonist, versus a placebo given 12 hours and 1 hour before a 200 mg levodopa dose. The study measured patients’ dyskinesia with the Rush Dyskinesia Disability Scale and found that nabilone, on average, decreased dyskinesia scores by 5 points and total LID time, while not interfering with levodopa efficacy. The other randomized crossover trial included 17 patients with LID severity of at least 2 based on the UPDRS. This study analyzed patient response to Cannador capsules, which contain 2.5 mg THC and 1.25 mg CBD, through change in UPDRS scores, as well as secondary measures that included the Rush scale and PDQ-39. Each patient took Cannador for four weeks and placebo for four weeks, with a two-week washout period in between. The study did not find any statistically significant change in scores with Cannador therapy and did not report any improvement in LID or other measures with Cannador [18].

Altogether, with the small sample sizes and inconsistent results of these studies, definitive conclusions cannot yet be drawn on
the efficacy of MC in PD treatment, which emphasizes the need for higher-quality research with larger samples.

**Methods**

**Study Methods**

This study is a retrospective chart review of patients from DENT Neurologic Institute’s Cannabis Clinic who were certified for medical cannabis under the New York State Medical Marijuana Program. The primary measure of this study was to determine the efficacy of medical cannabis in treatment of Parkinson’s disease patients in consideration of both motor and non-motor symptom management. Secondary measures of side effect profiles and dosing were also analyzed.

**Data Collection**

Patient files were identified and filtered from the DENT Cannabis Clinic New York State Medical Cannabis Certification Database. Data was collected using the secure, password-protected eClinical Works server used for all DENT patients. This information was recorded on a password-protected Excel spreadsheet to which only data abstractors had access. All chosen charts were assigned a research case number to protect patient information.

Patients were included in the study if they had a Parkinson’s disease diagnosis and were certified through the DENT Cannabis Clinic New York State Medical Cannabis Certification Database starting January 7, 2016. Patients also must have been compliant with MC for at least one month to be included.

Patients were excluded from the study if they did not have a Parkinson’s disease diagnosis, were not receiving cannabis under the New York State Medical Marijuana Program, or were not compliant with MC therapy for at least one month. Patients were also excluded if no follow-up data was present at the time of data analysis.

Efficacy data on patients’ responses to MC therapy was recorded as reduction in symptoms. Symptoms analyzed were tremor, spasticity, rigidity, gait improvement, dyskinesia, bradykinesia, pain, anxiety, nausea, sleep change, and mood change. If a patient reported reduction in a specific symptom, a “Yes” was documented. When a patient stated they did not experience reduction in a given symptom, a “No” was recorded. If the patient’s chart did not contain any information on a given symptom, an “NR” for “not recorded” was documented.

Information was also collected on patients’ MC doses as well as side effects. Dosage information included the product’s THC:CBD ratio, milliliters of tincture, inhalations of vapor or milligrams per capsule dose, and frequency of the dose taken daily. Whether patients experienced any side effects during MC therapy was listed as “Yes” or “No.” Specific side effects experienced during treatment were recorded, and the frequencies of each were analyzed. If the patient reported temporary side effects that receded with continued treatment, the side effects were recorded as “Transient.” However, for patients who reported continued side effects with treatment, side effects were recorded as “Not Transient.” Whether patients discontinued MC due to intolerable side effects was also documented as either “Yes” or “No.” A “Yes” was recorded if the patient stated they were unable to tolerate the side effects and therefore discontinued MC therapy.

Patients who were taking any natural, semi-synthetic, or synthetic prescription opioids at the time of MC initiation were recorded as “Yes” for opioid use. Then, any decrease in opioid use with MC therapy was documented as “Yes” in the category of changes in opioid consumption, and the new dose was compared to the old dose to calculate a percent reduction.

**Data Analysis**

Frequencies and percentages were calculated using data protected with research case numbers on Microsoft Excel.
Results

Out of 136 prospective patients, 62 (42 male, 20 female) patients aged 71±10 years met the inclusion criteria for this study. Most patients (69%) were excluded because they did not attend an MC follow-up visit at the time of analysis (Figure 4). PD was the primary indication for MC therapy in 44 patients (71%), while other primary indications were chronic pain in 16 patients (25%), cancer in 1 patient (2%), and neuropathy in 1 patient (2%).

![Figure 4: Exclusion Criteria Analysis](image)

In this sample, 77% (N=48) of patients reported an improvement in PD motor symptoms, which included spasticity (N=19), tremor (N=19), rigidity (N=13), abnormal gait (N=13), dyskinesia (N=5), and bradykinesia (N=3) (Figure 5). Of patients who reported on these symptoms, the frequencies of reported improvement are as follows: 95% for spasticity, 73% for tremor, 87% for rigidity, 72% for gait, and 75% for bradykinesia. All five patients who reported on dyskinesia noted improvement.

![Figure 5: Motor Symptom Improvement](image)
Over 75% of the sample complained of pain (N=47), and 85% (N=31) of this population reported decreased pain with MC therapy. Of the pain-afflicted population, 26 were taking an opioid at the initiation of MC therapy, and 54% of these patients either discontinued or reduced opioid use by more than 50% during their MC regimen. MC-mediated non-motor improvements were reported in 50% of the population and included improvements in sleep (N=18), anxiety (N=15), mood (N=11), and nausea (N=3) (Figure 6). Respectively, of patients who reported on these areas, the percentages who reported improvement are as follows: 78% for sleep, 75% for anxiety, and 69% for mood, and all three patients who reported on nausea noted improvement.

![Figure 6: Non-Motor Symptom Improvement](image)

Side effects were reported by 44% of patients, with the most common being somnolence (N=15), followed by confusion/disorientation (N=8) and dizziness (N=4) (Figure 7). Of the subset that reported side effects, side effects were transient in 41%. MC was tolerated well in this population, with only four patients (6.4%) discontinuing due to adverse effects. MC was most commonly prescribed in a 1:1 THC:CBD ratio in tincture formulation, with an average dose of 1.74 mL taken 2.5 times per day.
Discussion

This retrospective study is the first of its kind to evaluate the efficacy of MC in PD therapy in real-world clinical practice. Compared to other published research on cannabinoids and PD, this study has a large sample size. This research analyzed patients’ response to MC currently in use in over half of the U.S., rather than to synthetic cannabinoid preparations or recreational marijuana, to more accurately assess MC efficacy. Further, NYS has one of the most strictly regulated state MC policies that ensures standardized quality to all patients through obligatory safety testing of all MC for composition and contamination as well as exact calculation of THC and CBD milligram concentration in each product [1,19]. The design of this study required that the provider document patient improvement, which necessitated not only for the patient to report improvement but for the provider to objectively observe improvement. The study design, with direct provider follow up rather than patient surveys, also eliminated the bias that surveys often carry—that the patients who experience positive results are more likely to respond. With provider follow-up, if treatment is ineffective, patients are likely to still meet with their providers to consider other options.

This retrospective study on MC in 62 PD patients found improvement of 77% of the sample in PD motor symptoms and of 50% in evaluated non-motor symptoms. Therefore, MC exhibits efficacy in treating both motor and non-motor symptoms of PD. Given that all five patients who reported dyskinesia on levodopa therapy also described improvement indicates that MC may be effective in improving LID.

MC also proved successful in pain reduction, as 85% of patients who complained of pain reported pain relief with the MC regimen. Additionally, more than half of patients who began MC on an opioid reduced their opioid use by more than 50% or discontinued their opioid medications during their course of treatment, suggesting that MC may be effective in reducing opioid consumption. This result also supports the value of this therapy in opioid addiction management and potentially ameliorating the opioid crisis [20].

Though close to half of the sample reported side effects at some point in therapy, 41% only experienced transient side effects, and the most common side effects reported were...
somnolence, confusion, and dizziness. These side effects are of relatively low severity compared to those that may be experienced with use of other PD medications.

Together, these statistics support the efficacy of MC in PD treatment. As the majority of patients were also taking traditional PD medications while on MC, the results suggest that MC is likely an effective adjunctive therapy to traditional PD treatment regimens to help relieve refractory symptoms, treat non-motor symptoms, and/or alleviate side effects of PD medications.

The limitations to this study include the retrospective study limitations detailed previously, as well as the absence of scales such as the UPDRS, Rush scale, or PDQ-39 that would quantify the degree of improvement in symptomatology.

Conclusion

This retrospective study on MC use in 62 PD patients found MC effective and safe in treating both motor and non-motor symptoms of PD, mostly when used with other PD medications. The results also suggest potential efficacy of MC in alleviating a disagreeable effect of current PD therapy: levodopa-induced dyskinesia. Notably, a large majority of PD patients reported decreased pain with MC therapy, with more than half of the population on opioids either discontinuing or decreasing their use by at least 50% when on MC.

Though data was produced from retrospective patient chart analysis and carries the limitations of such a study, these promising results warrant future randomized, placebo-controlled trials that account for variables such as concomitant medications and may evaluate patients on objective rating scales such as the UPDRS, Rush scale, and PDQ-39. Such studies would verify the efficacy of MC in PD treatment in addition to the dosage information and side effect profiles. Additionally, long-term studies are necessary to investigate the neuroprotective effects that preclinical trials have found MC to enact in neurodegenerative disease as well as any other long-term effects of MC therapy.

References


[19] Title: Section 1004.11 – Manufacturing requirements for approved medical marijuana products. New York State Codes, Rules and Regulations Website.


Examining the Relationship Between Cortisol Levels, Environmental Factors, and Developmental Outcomes in Young Children

Amber McCranie1, Nahide Gungordu1, M.S., & Maria Hernandez-Reif1, PhD

Faculty Mentor: Maria Hernandez-Reif1, PhD

1Department of Human Development and Family Studies, The University of Alabama, Tuscaloosa, AL 35487

Cortisol is a hormone released in response to stress by the Hypothalamus-Pituitary-Adrenal (HPA) axis that acts on a multitude of complex pathways in the body contributing to the maintenance of homeostasis, metabolism, the flight-or-fight response, and more. This study examined the relationships between environmental factors, cortisol levels in children ages 0-2, and developmental outcomes. This study also aimed to determine a general trend in cortisol levels in children between the ages of 7.5 and 28 months. Infants and toddlers, their parents, and their childcare teachers participated in the study. Morning saliva samples were collected from young children every six months across the first two years of life. The saliva samples were assayed for cortisol levels. At these four points in time, parents completed a Daily Family Activities (DFA) questionnaire to examine their level of involvement with the children, while the teachers completed developmental profile assessments (DP-3) on the children. The measures completed by the parents and teachers were then scored and examined against standardized norms, as well as correlated with salivary cortisol levels. Higher cortisol levels were associated with greater stress responses. A positive correlation was found between the parent involvement scores at Time 1 and Time 2 and their child’s subsequent cortisol levels at Time 2 and Time 3, respectively, suggesting that parental involvement predicts children’s stress responses. Overall, children’s baseline cortisol levels were found to decline as they aged. Finally, there was a strong positive correlation found between children’s stress levels at Time 2 and their social-emotional developmental scores at Time 4. These findings are further discussed in the article, along with recommendations for future research.

Introduction

Cortisol is a glucocorticoid hormone released from the adrenal cortex in response to environmental stressors. This product is the result of a hormone cascade carried out by the Hypothalamus-Pituitary-Adrenal Axis (HPA). When exposed to a triggering event, the Hypothalamus releases Corticotropin Releasing Hormone (CRH), which acts on the Anterior Pituitary gland to release Adrenocorticotropic Hormone (ACTH). ACTH acts on the Adrenal Cortex in the kidneys to release cortisol. Cortisol is then able to enter the lipophilic plasma membrane of its target cell and bind to the glucocorticoid receptors in the cytoplasm [7]. There are cortisol receptors throughout all of the organ systems in the body working to maintain homeostasis; therefore, any fluctuations in cortisol production could vastly alter the body’s ability to operate properly [22].

Cortisol plays a large role in the stress response, and it also contributes to learning capacity, emotional expression, regulation of blood glucose, blood pressure, immunity, and more [21,22]. Maintaining moderate levels of cortisol is crucial for the development and preservation of many complex physiological functions. Several studies have found that extreme levels of cortisol, during the prenatal, infant, and early childhood periods are associated with diminished cognitive achievement and poor emotional regulation in children at later ages [2,6,7,17]. Children with chronic exposure to stress typically experience more difficulty with internalization of emotions and decreased peer interaction [2,19]. Extended periods of exposure to stressors can also lead to poor bodily health and increased susceptibility to disease [14].
The HPA axis and production of cortisol is influenced by and under the control of numerous bodily systems. For example, the circadian pacemaker in the hypothalamus regulates the activation of the HPA pathway according to one’s sleep schedule [19,20]. Cortisol levels are higher in the morning when first awakening and are often used to represent baseline levels of stress. Several studies have found that poor and fragmented sleep can be linked to fluctuations in normal cortisol levels [20]. This factor has led many to further research the correlation between cortisol levels and sleep cycles with the understanding that regular, high-quality sleep is a large determinant for managing stress.

Cortisol is a widely-regulated hormone and can become easily unbalanced by a variety of environmental factors such as sleep difficulty, caregiver attachment, temperament, childcare conditions, and peer interactions [2,3,9,11-16,19,20]. It also has the capacity to drastically affect the development of cognitive, adaptive, communicative, and social-emotional skills. Previous research has found that secure caregiver attachments, maternal connections in particular, buffer stress responses and moderate cortisol reactivity [9,15,16]. One study found that skin-on-skin contact between mothers and infants was correlated to decreased stress responses at later ages [15]. Another study found that prolonged feeding times with the caregiver were associated with a reduction in cortisol levels compared to brief feedings [13]. However, research has also found that overprotection of children by the mother, commonly referred to as “helicopter parenting,” is linked to children exhibiting higher stress reactivity and greater sleep problems [11,12]. This effect could be moderated by interventions involving the mother encouraging independence and utilization of emotional regulation techniques [11]. Common to all of these studies is the finding that caregivers play a critical role in young children’s cortisol fluctuation and maturation of their HPA axis, or stress response system.

There have been many studies about stress concerning adolescent and adult populations, but there has not been extensive research into the role that stress plays in children between the ages of 7.5 and 28 months. The current study examined the relationships between children’s home environment as reported by parents, young children’s morning cortisol levels, and children’s developmental milestones as reported by teachers. Based on this literature review, there should be a general decrease in cortisol levels across infancy and toddlerhood, paralleling HPA axis maturation and general development. There is also expected to be a relationship between cortisol levels and parent involvement, as well as a relationship between cortisol levels and social-emotional development.

Method

Participants

26 infants, about 7.5 months of age (Mean age = 7.88, SD = 2.51) at the time of recruitment, their parents, and their teachers participated in the study. The infants were enrolled in a childcare program in a medium-size town, in a southern state in the United States. Data were collected four times over a two-year period, every six months starting at the time of recruitment. Table 1 displays demographic data about the children and parents. The following measurements and information were obtained related to the children.

<table>
<thead>
<tr>
<th>Participants’ profile</th>
<th>Mean ±SD or % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age T1 Children</td>
<td>7.88 ± 2.51</td>
</tr>
<tr>
<td>Age T2</td>
<td>14.00 ± 3.22</td>
</tr>
<tr>
<td>Age T3</td>
<td>21.00 ± 3.72</td>
</tr>
<tr>
<td>Age T4</td>
<td>28.00 ± 4.06</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Girl</td>
<td>53.8 % (n=14)</td>
</tr>
<tr>
<td>Boy</td>
<td>46.2 % (n=12)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>100 % (n=26)</td>
</tr>
<tr>
<td>SES</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>57.7 % (n=15)</td>
</tr>
<tr>
<td>Upper</td>
<td>34.6 % (n=9)</td>
</tr>
<tr>
<td>Middle</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>7.7 % (n=2)</td>
</tr>
</tbody>
</table>

Table 1. Demographic Descriptive Statistics

N=26. T: Time, SES: Socioeconomic Status. Age is noted in months.
Salimetric children’s swabs were used to collect the infants’ morning saliva levels at their childcare program [18]. A single swab was inserted into the child’s mouth, and the child was allowed to chew on the swab for a full minute, or until they provided a sufficient amount of saliva for assaying. The child’s swab was then placed in a tube and labeled according to the child’s subject number, cohort, time, and date. In order to ensure reliable data and attempt to moderate the confounding variable of the child being stressed as a result of the saliva collection, second and third saliva samples were collected using the same procedure, each at 20-minute intervals. These samples could then be assessed to reveal infants’ cortisol reactivity to an unfamiliar adult collecting their saliva. In all, saliva collection occurred between 8 am and 9 am to ensure the child’s stress levels most accurately reflected a basal measurement and were consistent across measuring times, as well as across participants. After the three saliva samples were collected, they were stored in a subzero industrial freezer until they were sent for assaying. The samples relevant to this study will be referred to as Time 1 (Mean age = 7.88), Time 2 (Mean age = 14.00), Time 3 (Mean age = 21.00), and Time 4 (Mean age = 28.00). Higher cortisol levels reflected higher stress hormone levels. Previous data suggests that cortisol measurements by means of saliva yield similar results to those obtained by blood serum and thus are reliable, as well as less traumatic for young children [8].

Home Environment Questionnaire

The level of parent involvement and aspects of the home environment were measured by a Daily Family Activities (DFA) questionnaire designed specifically for this study. Within the DFA, the parents were asked to answer multiple questions pertaining to their child’s 1) sleep patterns, 2) mealtime, 3) leisure activities, and 4) prosocial skills. Each question was recorded and then recoded according to standard values, then added together to provide totals in each of the categories listed above. Several values were then regrouped into a new category of Parent Involvement, and the scores were totaled again.

Developmental Profile

The teacher’s perception of the child’s development was measured through the Developmental Profile (DP-3) [1]. The DP-3 is designed to reflect skill development from birth to 12 years and 11 months [1]. The DP-3 consists of a series of questions divided into five categories: 1) physical, 2) adaptive behavior, 3) cognitive, 4) social-emotional, and 5) communication. The number of consecutive “yes” answers related to children’s progressive skills in each area were totaled to give a raw score and standardized for comparison. The standard score in each category was then used to determine if the child’s development was on track for their age, the percentile the child fell in, and the age equivalence with which the child’s abilities coincided. The child’s standardized scores were also added and further recoded to provide a general development score, percentile ranking, and age equivalence to obtain group means.

Data Analysis

Correlation coefficients (r) were determined by relating each of the variables outlined above in the DP-3 and DFA to the assayed cortisol levels. A p-value of <0.05 was used to indicate a significant relationship. An r-value of >0.5 was used to indicate a strong positive correlation.

Results

Parent Involvement

A positive correlation was found between parent involvement scores from the DFA at Time 1 and Time 2 and children’s cortisol levels at Time 2 and Time 3. Parent involvement at Time 1 was moderately correlated with children’s cortisol levels at Time 3; r=0.50 and p=0.02 (n=20). There was also a statistically significant relationship between parent involvement scores at Time 2 and cortisol levels in the last two saliva samples (B and C) taken at Time 2. This time period reflects the one-year mark, or around the beginning of toddlerhood. The first saliva sample (A) taken at Time 2 was not significantly related to any variable. However, the second saliva sample (B), taken 20 minutes after the first, resulted in an r-
value of 0.52, and $p<0.05$ (n=20). The third saliva sample (C), taken 40 minutes after the first, resulted in an r-value of 0.48, and $p<0.05$ (n=18).

**Cortisol Trend in Young Children**

The mean cortisol levels of children as a group were found to generally decrease from birth to two years, with the highest cortisol levels detected between birth to around 14 months. Figure 1 displays the trend in cortisol levels.

**Figure 1.** Cortisol levels across the first two years of life

**Social-Emotional Development**

There was a significant relationship found between all three samples of the children’s cortisol levels at Time 2 (toddlerhood) and their social-emotional development scores from the DP3 at Time 4 (2-year milestone). The samples revealed r-values of 0.660, 0.691, and 0.644 with $p$-values of <0.001, <0.001, and <0.05, respectively.

**Discussion**

Like most aspects of human development, parenting extremes in any capacity can result in developmental delays or challenges at later ages, so moderation is key. Infants and younger children rely on their parents for basic needs, so their stress levels are in turn highly variable based on their feelings of security and attachment [2,7,9,14,15,16]. Previous research has found that children with insecure attachments to their mothers were more likely to have elevated levels of cortisol at 18 months [16]. Further research would be required, but numerous explanations could produce the positive relationship found here between the parent involvement scores at Time 1 and Time 2 and the cortisol levels at Time 2 and Time 3. One explanation could be centered around the child experiencing immense expectations or frustration when attempting to develop autonomy and independence, as a result of “helicopter parenting.” This explanation aligns with the previously-discussed study that found that maternal protection led to more nervous behaviors down the line for children with high stress sensitivity [11]. In that study, this increase was able to be moderated by encouragement of self-sufficiency [11]. These results are meaningful because warnings often focus on neglect and child abuse as being solely harmful to development; however, this result reveals there are adverse effects from the other parenting extremes as well. Data suggests that nurturing, stimulating, and stable households enhance HPA regulation and give children better opportunities to handle stressful situations [14]. Another explanation could be that in the first few years of life, the HPA axis is immature and still developing as the child adapts to his or her unique environment. Further research can help determine which of these two explanations may clarify the current findings.

The general trend of decreasing cortisol levels from around the middle of the first year to the end of the second year of life was expected, based on the results of previous studies and literature. As children grow, they are scheduled to complete a number of developmental milestones that lead to overall greater autonomy and higher-level cognitive functioning that could possibly account for subsequent reductions in stress. For example, between nine to 12 months of age, children begin to develop more complex communication skills: responding to their name, forming small words, and using their hands to gesture [5]. This milestone may reduce the stress that a child feels attempting to understand their situation or communicate their needs to caregivers. Between seven to 12 months, children also begin transitioning from crawling to standing and walking [5], which allows children to exert greater independence over their surroundings. One study found similar results to those here: cortisol reactivity naturally decreased with age as children’s stressors became more psychological than physical and development of
the HPA axis left the child better able to buffer adversity [10]. Understanding the mechanisms of a child shifting their dependence from a caregiver to themselves, in many aspects, is critical to understanding the underlying causes of stress that can lead to both positive and negative long-term effects.

One final relationship determined was a correlation between higher cortisol levels at Time 2 and higher raw social-emotional scores at Time 4. This relationship revealed in the study was strong, as all three saliva samples tested at Time 2 fell above an r-value of 0.600 and below a p-value of 0.05. In previous studies and literature reviews, higher levels of cortisol have been found to be related to greater internalization of emotion and poorer emotional regulation, which seemingly contrasts the result found here of higher stress levels leading to greater social-emotional scores [2,6,19]. Other reviews found that dysregulation of the HPA axis and greater baseline levels of cortisol were more often associated with unstable relationships [14]. Overall, the data reviewed typically supported social interaction cyclically affecting and being affected by a child’s ability to control their emotions. For example, children who struggle with social interaction as a result of poor emotional regulation may be rejected by their peers and in turn have higher levels of cortisol and more negative emotion [14,20]. One study revealed a possible explanation for the opposing result found here. It determined that a relationship existed among inflated cortisol levels resulting from exposure to stress-inducing events, enhanced memory processing, and long-term memory recall [4]. Another explanation could be that in the first year of life, infant cortisol reactivity to interactions with an unfamiliar adult in a childcare program, which is considered a stressor, reflects an immature HPA axis. Stranger anxiety emerges around seven to nine months of age and is prevalent until 18 months or older. Perhaps the reactivity in the first year of life is related to a reaction to strangers in the childcare center, or higher infant cortisol levels are normal and simply a sign that the HPA axis is attempting to respond to and regulate stress. This would be followed by the observed decrease in cortisol over the second year of life as a toddler’s HPA axis becomes more mature and better regulated. Future studies are needed to replicate these findings.

**Limitations**

This study was intended to cast a broad net across home environment, cortisol levels, and developmental scores to determine if any relationships existed. While there were several statistically significant relationships discovered, some aspects of the study make the results difficult to generalize. In particular, this study utilized a small sample size and was restricted to the children attending a middle- to upper-class, high-quality childcare program. The sample is also constrained to one race; thus, the demographics may not be representative and further, more diverse studies would be required to generalize the results to other populations. This study was also limited in scope, as only correlational analysis was conducted, and causational analysis would require separate, more in-depth experimentation, focused on one particular result rather than a comprehensive review. The data collection by means of questionnaires could possibly skew the results, as answers may not be accurate or honest on behalf of the parent or teacher due to social-desirability or acquiescence biases. Another possible limitation of this study is the accuracy of cortisol measurements. Three saliva samples were collected at each time point, 20 minutes apart, to ensure precision, and allow for the lag of cortisol being reflected in the saliva sample. However, the samples were not collected over several days and could thus be less representative of typical cortisol levels if the child was overstimulated on the day of testing due to extraneous circumstances.

**Future Recommendations**

The results of this study could prompt future research on infants’ cortisol responses in relation to their environment. Research focused on the direct relationship between the specific aspects of high parent involvement that could lead to high cortisol levels would be beneficial. Another study that attempts to replicate the results found here with a positive relationship between stress levels and social-emotional scores would also be useful, along with a deeper analysis of the explicit factors that could
produce such a relationship. Finally, the results of this study could be influential in the discussion of intervention programs for moderating stress in infancy and early toddlerhood.

References


Differences in Physiologic Measures of Infant Swallowing Function for Swallows with Varying Degrees of Airway Invasion

Sarah G. Trulove¹, Bailee C. Gist¹, Memorie M. Gosa, Ph.D., CCC-SLP, BCS-S¹,²

Faculty Mentor: Memorie M. Gosa, Ph.D., CCC-SLP, BCS-S¹

¹Department of Communicative Disorders, The University of Alabama, Tuscaloosa, AL 35487
²LeBonheur Children's Hospital, Memphis, TN 38103

Swallowing disorders (dysphagia) in pediatric populations are a serious threat to achieving nutritional intake. Due to the implications of pediatric dysphagia, accurate diagnosis and effective treatment are necessary for avoiding nutritional and medical sequelae. This study utilized retrospective frame-by-frame analysis to collect eight measures of infant swallowing function from two consecutive swallows that demonstrated varying degrees of airway invasion extracted from a convenience sample of 10 infant (< 4 months of age) videofluoroscopic swallow studies. Videofluoroscopic swallow studies were completed at a comprehensive regional pediatric hospital in an urban location. Degree of airway invasion was measured using Penetration-Aspiration Scale scores. Penetration-Aspiration Scale scores were then used to categorize swallows as having less airway invasion (Swallow 1) and more airway invasion (Swallow 2), and comparisons of the eight physiologic measures were made between Swallow 1 and Swallow 2. This pilot study found that there were quantifiable differences in five of the eight measures of infant swallow function, but the differences did not reach statistical significance. This study demonstrates for the first time the variability within infant swallowing function between swallows of varying degrees of airway compromise. Future research should focus on the five measures that revealed median changes between swallows of varying degrees of airway invasion with a larger participant pool to determine if those differences are statistically significant.

Introduction

Safe and efficient swallowing ensures that infants receive the necessary nutrition and hydration to support growth and maturation [1]. The development of swallow coordination begins in the fetal period. Consistent swallowing can be observed as early as 22 to 24 weeks of gestation, with suck-swallow-breathing coordination being established by 32 to 34 weeks of gestation [2]. This coordination further matures between 33 and 36 weeks of gestation, resulting in consistent suck-swallow-breathing coordination by 37 weeks of gestation [2,3]. Likewise, most infants have sufficient suck-swallow-breathing coordination by 34 weeks of gestation to begin bottle-feeding and breast-feeding [4]. Infants continue to rely on a liquid diet of breastmilk or formula to supply them with all the necessary micro and macro nutrients to support growth and development through the first year of life [5]. A purely liquid diet puts infants with dysphagia at risk to more readily experience laryngeal penetration and/or aspiration during a swallow [6].

Swallowing disorders (dysphagia) in pediatric populations are a serious threat to achieving adequate nutritional intake [7]. Dysphagia is a known comorbidity of many common pediatric medical conditions including neurologic diseases and disorders, prematurity, cardiopulmonary diseases and disorders, and craniofacial anomalies [8]. Dysphagia in pediatric populations can result in growth faltering, difficulties with transitioning to consuming solids, respiratory distress, recurrent upper respiratory infections, increased gagging, orofacial hypersensitivity, food refusal, or any combination of outcomes [9]. Due to the serious health implications of pediatric dysphagia, accurate diagnosis and effective treatment are necessary for avoiding nutritional and medical sequelae [10].

The videofluoroscopic swallowing study (VFSS) is a commonly-used radiographic
imaging technique for evaluating swallowing function in infants. VFSS allows for visualization of a bolus as it moves through the mouth, pharynx, and upper esophagus during the oral, pharyngeal, and esophageal phases of swallowing [8]. Additionally, there are reliable, previously-established measures of swallowing function that can be derived from review of VFSS that allow for objective description of infant swallowing function [11-15]. These measures include number of sucks per swallow, suck time in seconds, oral transit time in seconds, pharyngeal transit time in seconds, duration of cricopharyngeal opening in seconds, duration of pharyngeal constriction in seconds, time to achieve laryngeal closure in seconds, duration of laryngeal closure in seconds, Penetration-Aspiration Scale scores, bolus location, presence of residue, location of residue, jaw position during the pharyngeal phase of swallow, presence of epiglottic tilt, and presence of nasopharyngeal backflow [11-14]. The clinical utility of these measures to determine the cause of airway invasion events and to guide the application of compensatory strategies has not been established.

Previous studies have noted that swallowing function in infants with and without dysphagia changes over time [1,10,11]. For infants with dysphagia, this means that within a single feeding there can be safe swallows (those without any airway compromise events) and unsafe swallows [10,11]. Previous analyses of infant swallowing function with VFSS documented the co-occurrence of swallows with varying degrees of airway invasion during a continuous swallowing sequence [16]. The temporal and physiologic differences in swallows that result in varying degrees of airway invasion (ranging from no airway invasion to aspiration with no cough or clear from the subglottic region) have not been described in the existing literature. Therefore, in this retrospective pilot study, the authors sought to determine if measures of infant swallowing function that have been previously shown to be reliable could be used to illustrate quantifiable differences between swallows that demonstrate varying degrees of airway invasion (as measured by the Penetration-Aspiration Scale) [17-19]. It is important for clinicians to be able to accurately discern not only the presence of airway compromise events, but also the physiologic breakdown in swallowing function that led to the airway compromise event by analyzing the VFSS. Knowledge of the physiologic change that results in airway compromise during swallowing is necessary to recommend appropriate compensatory strategies to allow for improved safety and efficiency of swallowing function when possible. The authors hypothesized that these previously-reported measures of infant swallowing function would show significant differences between swallows with varying degrees of airway invasion.

Materials and Methods
This study involved a secondary analysis of an existing dataset of archived infant VFSS video recordings. The original dataset was derived from a retrospective review of 20 infant VFSS that were randomly selected from a potential participant pool of VFSS performed at a regional pediatric hospital in 2008. All VFSS performed at the pediatric hospital in 2008 were completed at a pulse rate of 30 pulses per second (continuous VFSS). Clinical standards of the facility require that infants undergoing VFSS are seated, semi-upright, in a Tumbleform (Fabrications Enterprises Inc., Elmsford, NY) chair and are viewed in the lateral projection. Each infant is presented with Varibar Thin Liquid Barium (Bracco Diagnostics Inc, Monroe Township, NJ) (target viscosity of 4 centipoise, viscosity range <15 centipoise) first and then may be presented with compensatory, thickened liquid bariums if swallowing function observed indicates its administration. All liquids are prepackaged with standard viscosity targets and manufactured by Bracco Diagnostics Inc. (Monroe Township, NJ). The thin liquid barium requires reconstitution from powder form, and clinicians follow the manufacturer’s instructions to prepare standard thin liquid barium fluid, which controls for any variability in liquid consistency presented. Liquids were offered from a Similac (Abott Laboratories, Abott Park, IL) disposable volu-feeder bottle with standard Similac (Abott Laboratories, Abott Park, IL), disposable one-hole nipple. Only swallows of the Varibar Thin Liquid Barium (Bracco...
Diagnostics Inc, Monroe Township, NJ) were included for analysis.

For inclusion in the original participant pool, infants had to be between birth and four months of age and have moderate-severe oropharyngeal dysphagia based on clinical review of VFSS by a qualified pediatric speech-language pathologist with extensive training in the administration and interpretation of pediatric VFSS. The clinical impression of moderate-severe oropharyngeal dysphagia indicated that participants demonstrated breakdown in the safety and efficiency of oropharyngeal swallowing function in one or more of the traditional swallowing phases (oral, pharyngeal, and/or esophageal) that resulted in episodes of laryngeal penetration and/or aspiration recorded during their study. The original VFSS studies were reviewed by a speech-language pathologist with more than 15 years of experience in administering and interpreting infant VFSS with the clinical distinction of Board-Certified Specialist in Swallowing and Swallowing Disorders to document the presence of laryngeal penetration and/or aspiration in each video. A clip that contained three to five swallows was then extracted from the original VFSS. The extracted clip included at least one swallow before and after the swallow that contained the airway invasion event (either laryngeal penetration and/or aspiration). In the original study, the swallow of interest was the swallow that contained the airway invasion. In review of the full clip, analysts noted that for 50% (N=10/20) of the participants included in the original study, there was a difference in the airway invasion severity between swallows within a single suckling burst. Those 10 participants with differences in airway invasion severity between swallows were then chosen for follow-up analysis in this study to attempt to determine the cause of variability in airway invasion between swallows in the same suckling sequence. All participants were de-identified and coded for review in both studies to protect privacy. Institutional Review Board (IRB) approval was received from The University of Alabama and The University of Tennessee Health Science Center prior to study initiation.

The data set included in this study consisted of the deidentified footage of those 10 infants (≤4 months of age) from the original study that demonstrated variability of airway invasion events within the original extracted clip. A sequence of two continuous swallows during which the infant had one swallow with more airway invasion and one swallow with less airway invasion during the suckling burst was isolated for further analysis. The files were saved in an audio video interleave (AVI) format prior to analysis by independent reviewers. The videos were reviewed in a randomly-assigned order. Two research assistants (reviewer one and reviewer two) with previous training in analysis of infant VFSS were assigned to independently analyze the videos. Reviewer one watched all of the videos, and reviewer two watched 40% of the videos to provide inter-rater reliability. The reviewers were blinded to each other’s ratings. Reviewer one waited two weeks and then blindly reviewed 40% of the videos again to establish intra-rater reliability.

Reviewer one and reviewer two underwent four weeks of reliability training during hour-long sessions once per week with follow-up practice during the following week for accurate identification of established temporal and physiologic measures of infant swallowing function outlined in Table 1 [12].
Table 1: Instructions for determination of physiologic measures  

<table>
<thead>
<tr>
<th>Physiologic Measure</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetration-aspiration</td>
<td>Assigned a number, 1-8, from the penetration-aspiration scale to describe the amount of airway invasion during the swallow</td>
</tr>
<tr>
<td>Suck time</td>
<td>Begin with frame at initiation of downward mandibular movement and end with frame at initiation of base of tongue propulsion. The difference between these two measures was the time spent sucking</td>
</tr>
<tr>
<td>Oral transit time</td>
<td>Begin with frame at initiation of base of tongue propulsion and end with last frame where body of bolus material is in the valleculae. The difference between these two measures was the oral transit time</td>
</tr>
<tr>
<td>Number of sucks per swallow</td>
<td>Downward motion of mandible-to-mandible returning to neutral position was counted as one suck. Total number of sucks per swallow was counted</td>
</tr>
<tr>
<td>Pharyngeal transit time</td>
<td>Begin with last frame where body of bolus material is in the valleculae and end with last frame of cricopharyngeal opening. The difference between these two measures was the pharyngeal transit time</td>
</tr>
<tr>
<td>Time to laryngeal closure</td>
<td>Begin with first frame at initiation of laryngeal closure with upward movement of the arytenoids and end with first frame of complete laryngeal closure. The difference between these two measures was the time to laryngeal closure</td>
</tr>
<tr>
<td>Pharyngeal constriction</td>
<td>Begin with first frame of maximum pharyngeal constriction and end with onset of pharyngeal relaxation at the velum. The difference between these two measures was the duration of pharyngeal constriction</td>
</tr>
<tr>
<td>Cricopharyngeal opening</td>
<td>Begin with first frame of bolus head in the cricopharyngeal sphincter and end with first frame where cricopharyngeal sphincter is closed and bolus tail has entered esophagus. The difference between these two measures was the duration of cricopharyngeal opening</td>
</tr>
</tbody>
</table>
A clinical pediatric speech-language pathologist with more than 15 years of experience in administering and interpreting results from infant VFSS and clinical distinction of Board-Certified Specialist in Swallowing and Swallowing Disorders provided training for the selected swallowing measures. Training began by rating measures from adult VFSS on commercially-available training packages and then transitioned to practice rating measures on de-identified pediatric VFSS that were not part of the data set for this project. Videos were reviewed utilizing QuickTime software (Apple, Cupertino, CA) with frame-by-frame analysis as many times as necessary for reviewers to feel confident in their ratings. Prior to beginning this project, the two independent reviewers achieved high percent agreement (90% or greater) for intra-rater reliability and for inter-rater reliability with each other and with the expert speech-language pathologist for all of the measures (Penetration Aspiration Scale, suck time, oral transit time, number of sucks per swallow, pharyngeal transit time, time to laryngeal closure, pharyngeal constriction duration, and cricopharyngeal opening duration) collected for this study.

Once a high level of inter- and intra-rater agreement was achieved through training and practice, independent reviewer one analyzed the two preselected swallows for each participant (N=10 participants, total of 20 swallows reviewed) included in this study in a randomly-assigned order (see Table 1 for description of measures collected during review). After measures were collected, the Penetration-Aspiration Scale (PAS) scores [17-19] (shown in Fig. 1) were used to differentiate the varying degrees of airway invasion between the two swallows for comparison. The Penetration Aspiration Scale (PAS) was used to measure airway compromise events. The PAS assigns a value of one to eight to each swallow to indicate the degree of airway invasion (none (1), laryngeal penetration (2-5), or aspiration (6-8)) observed during the swallow [17]. The eight-point PAS approximates ordinality and intervality, allowing for statistical analysis requiring interval-level variables [18]. It has also been shown to be a reliable tool for describing penetration and aspiration events in pediatric populations, including infants [19,12].
The collected measures from each participant were divided into two groups based on severity of airway invasion of each swallow as determined by PAS scores: Swallow 1 represented the measures of the swallow with less airway invasion and had PAS scores of 1 (no airway invasion) or 2 (laryngeal penetration above the level of the vocal folds that clears the laryngeal vestibule after the swallow), and Swallow 2 represented the measures of the swallow with more airway invasion and had PAS scores ranging from 2 to 8 (aspiration with no cough or attempt to clear aspirated material).
IBM Statistical Package for the Social Sciences (SPSS) for Mac version 25 (IBM Corp., Armonk, NY) was used for statistical analyses. An intraclass correlation coefficient was used to determine inter- and intra-rater reliability for the swallowing measures collected by the two raters [20]. A series of Wilcoxon signed-rank tests were conducted to determine if there were statistically significant median changes in physiological features of swallowing function (suck time, oral transit time, number of sucks per swallow, pharyngeal transit time, time to achieve laryngeal closure, duration of laryngeal closure, pharyngeal constriction duration, and duration of upper esophageal sphincter opening) between Swallow 1 and Swallow 2. The different scores were distributed symmetrically as assessed by a histogram with a superimposed normal curve. Nonparametric statistical analyses were chosen to determine if quantifiable differences in measures of swallowing function existed between Swallow 1 and Swallow 2 due to the small sample size of this pilot project (N=10).

Results

There was excellent inter- and intra-rater reliability of the data set as established by intraclass correlation coefficient (ICC) analysis. The ICC for inter-rater reliability for difference scores calculated for all measures between Swallow 1 and Swallow 2 was excellent, with ICCs ranging from 0.99 to 1.00. The ICCs for intra-rater reliability between reviewer one’s first and second viewing of a randomly selected 40% sample for all measures of swallow function collected for Swallow 1 and 2 had ICCs ranging from 0.99 to 1.00.

The demographic information of the 10 participants is provided in Table 2. The PAS scores and oral measures of swallowing function for Swallow 1 and Swallow 2 for each participant are presented in Table 3.
### Table 2: Demographic information of participants

<table>
<thead>
<tr>
<th>Participant no.</th>
<th>Age in weeks</th>
<th>Gender</th>
<th>Primary Diagnosis</th>
<th>Reason for Referral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>F</td>
<td>Respiratory, Gastrointestinal</td>
<td>Rule out dysphagia</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>M</td>
<td>None</td>
<td>Coughing, choking, gagging with feeding</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>M</td>
<td>Respiratory</td>
<td>Rule out dysphagia</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>M</td>
<td>Neurological, Metabolic</td>
<td>Rule out dysphagia</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>M</td>
<td>Respiratory, Gastrointestinal</td>
<td>Choking with feeding</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>M</td>
<td>Respiratory, Gastrointestinal</td>
<td>Choking with feeding</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>M</td>
<td>History of prematurity, Respiratory</td>
<td>Rule out dysphagia</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>F</td>
<td>History of prematurity, Cardiac, Genetic</td>
<td>Oxygen desaturation with feeding</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>M</td>
<td>History of prematurity, Respiratory, Cardiac</td>
<td>Rule out dysphagia</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>M</td>
<td>Renal</td>
<td>Wheezing with feeding</td>
</tr>
</tbody>
</table>

*Note: no. = Number; F = Female; M = Male*
Table 3: PAS scores and oral measures for swallow 1 and swallow 2

<table>
<thead>
<tr>
<th>Participant</th>
<th>PAS S1</th>
<th>PAS S2</th>
<th>Suck Time S1</th>
<th>Suck Time S2</th>
<th>Swallow S1</th>
<th>Swallow S2</th>
<th>Oral Transit S1</th>
<th>Oral Transit S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.92</td>
<td>2.10</td>
<td>1</td>
<td>2</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>8</td>
<td>0.96</td>
<td>0.26</td>
<td>1</td>
<td>1</td>
<td>0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1.06</td>
<td>1.07</td>
<td>2</td>
<td>2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>1.08</td>
<td>1.79</td>
<td>1</td>
<td>1</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0.92</td>
<td>0.96</td>
<td>1</td>
<td>1</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1.50</td>
<td>1.87</td>
<td>2</td>
<td>3</td>
<td>0.30</td>
<td>0.23</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4</td>
<td>0.30</td>
<td>0.14</td>
<td>1</td>
<td>1</td>
<td>0.13</td>
<td>0.26</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>8</td>
<td>0.73</td>
<td>0.87</td>
<td>1</td>
<td>1</td>
<td>0.97</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>4</td>
<td>0.87</td>
<td>1.01</td>
<td>1</td>
<td>2</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>3</td>
<td>0.95</td>
<td>1.02</td>
<td>1</td>
<td>1</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: S1 = Swallow 1 (least airway invasion); S2 = Swallow 2 (most airway invasion); PAS = Penetration aspiration scale score (1-8); Suck time and oral transit time reported in seconds; Sucks/swallow = number of sucks per swallow.
The PAS scores and pharyngeal measures of swallowing function for Swallow 1 and Swallow 2 for each participant are presented in Table 4.

### Table 4: PAS scores and pharyngeal measures for swallow 1 and swallow 2

<table>
<thead>
<tr>
<th>Participant</th>
<th>PAS S1</th>
<th>PAS S2</th>
<th>PTT S1</th>
<th>PTT S2</th>
<th>LC S1</th>
<th>LC S2</th>
<th>LCD S1</th>
<th>LCD S2</th>
<th>PCD S1</th>
<th>PCD S2</th>
<th>CP S1</th>
<th>CP S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.06</td>
<td>0.07</td>
<td>0.03</td>
<td>0.08</td>
<td>0.92</td>
<td>0.93</td>
<td>0.12</td>
<td>0.86</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>8</td>
<td>0.78</td>
<td>0.10</td>
<td>0.71</td>
<td>0.02</td>
<td>0.14</td>
<td>0.91</td>
<td>0.79</td>
<td>0.86</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.80</td>
<td>0.11</td>
<td>0.06</td>
<td>0.07</td>
<td>0.86</td>
<td>0.90</td>
<td>0.77</td>
<td>0.11</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>0.76</td>
<td>0.09</td>
<td>0.06</td>
<td>0.02</td>
<td>0.93</td>
<td>0.92</td>
<td>0.83</td>
<td>0.12</td>
<td>0.76</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0.07</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
<td>0.18</td>
<td>0.21</td>
<td>0.12</td>
<td>0.13</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0.34</td>
<td>0.27</td>
<td>0.50</td>
<td>0.20</td>
<td>0.53</td>
<td>0.40</td>
<td>0.20</td>
<td>0.17</td>
<td>0.37</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4</td>
<td>0.37</td>
<td>0.34</td>
<td>0.20</td>
<td>0.17</td>
<td>0.74</td>
<td>0.87</td>
<td>0.30</td>
<td>0.20</td>
<td>0.26</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>8</td>
<td>0.20</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.87</td>
<td>0.74</td>
<td>0.66</td>
<td>0.30</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>4</td>
<td>0.08</td>
<td>0.79</td>
<td>0.04</td>
<td>0.02</td>
<td>0.83</td>
<td>0.85</td>
<td>0.06</td>
<td>0.78</td>
<td>0.07</td>
<td>0.76</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>3</td>
<td>0.09</td>
<td>0.08</td>
<td>0.02</td>
<td>0.06</td>
<td>0.93</td>
<td>0.93</td>
<td>0.15</td>
<td>0.84</td>
<td>0.09</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Note:** PAS= Penetration aspiration scale score (1-8); S1= Swallow 1 (least airway invasion); S2= Swallow 2 (most airway invasion); PPT= Pharyngeal transit time reported in seconds; LC= Time to laryngeal closure reported in seconds; LCD= Duration of laryngeal closure reported in seconds; PCD= Pharyngeal constriction duration reported in seconds; CP= Cricopharyngeal sphincter opening duration reported in seconds

Review of the raw data reveals differences in several of the measures as compared between Swallow 1 and Swallow 2. In consideration of measures taken during the oral phase of swallowing (time spent sucking, number of sucks per swallow, and oral transit time) the data reveal that only three participants had any change in the number of sucks per swallow utilized between Swallow 1 and Swallow 2 and that those three participants only utilized one additional suck during Swallow 2 as compared to Swallow 1. The majority of the infants utilized the same number of sucks per swallow between swallows of more airway invasion and less airway invasion. The majority of swallows that resulted in greater airway invasion (Swallow 2 with range of PAS scores from 2 (N=4) to 8 (N=3)) had longer sucking times than those swallows that resulted in less airway invasion. Finally, review of the difference in oral transit times between Swallow 2 and Swallow 1 reveals a mixed pattern, with three oral transit times being longer for Swallow 1, four oral transit times being longer for Swallow 2, and three showing no change in oral transit times between Swallow 1 and Swallow 2.

In consideration of measures taken during the pharyngeal and esophageal phases of swallowing (pharyngeal transit times, time to reach laryngeal closure, duration of laryngeal closure, pharyngeal constriction duration, and cricopharyngeal sphincter opening duration),
there are several patterns of differences to consider. First, considering pharyngeal transit times, seven of the participants demonstrated longer pharyngeal transit times for Swallow 1 (less airway invasion) as compared to Swallow 2 (more airway invasion). However, for measures of differences in the duration of laryngeal closure, duration of pharyngeal constriction, and duration of cricopharyngeal sphincter opening, the majority of the values recorded were longer for Swallow 2 as compared to Swallow 1. Finally, for differences in time to reach laryngeal closure, there was a mixed pattern, with five participants demonstrating longer time to reach laryngeal closure during Swallow 1, three participants demonstrating longer time to reach laryngeal closure during swallow 2, and two participants demonstrating no change in time to reach laryngeal closure between the two swallows.

Despite observable differences in the raw data, when comparing the physiologic measures of swallowing function, none of the measures achieved statistically significant median changes between Swallow 1 and Swallow 2. The results are summarized in Table 5.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Diff PAS</th>
<th>Diff Suck Time</th>
<th>Diff Sucks/Swallow</th>
<th>Diff OTT</th>
<th>Diff PTT</th>
<th>Diff LC</th>
<th>Diff LCD</th>
<th>Diff PCD</th>
<th>Diff CPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1.18</td>
<td>1</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.74</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-0.70</td>
<td>0</td>
<td>0.72</td>
<td>-0.68</td>
<td>-0.69</td>
<td>0.77</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.01</td>
<td>0</td>
<td>0.00</td>
<td>-0.69</td>
<td>0.01</td>
<td>0.04</td>
<td>-0.66</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.71</td>
<td>0</td>
<td>0.00</td>
<td>-0.67</td>
<td>-0.04</td>
<td>-0.01</td>
<td>-0.71</td>
<td>-0.68</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.04</td>
<td>0</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.37</td>
<td>1</td>
<td>-0.07</td>
<td>-0.07</td>
<td>-0.30</td>
<td>-0.13</td>
<td>0.17</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-0.16</td>
<td>0</td>
<td>0.13</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0.13</td>
<td>0.09</td>
<td>0.33</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>0.14</td>
<td>0</td>
<td>-0.44</td>
<td>-0.10</td>
<td>0.00</td>
<td>-0.13</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0.14</td>
<td>1</td>
<td>0.00</td>
<td>0.71</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.72</td>
<td>0.69</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0.07</td>
<td>0</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.04</td>
<td>0.00</td>
<td>0.69</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

Median Difference: 0.11  0.00  0.00  -0.05  -0.01  0.02  0.13  0.04

z-score: 1.37  1.73  0.72  -1.53  -0.95  0.92  1.38  1.48

p-value: 0.17  0.08  0.47  0.13  0.34  0.36  0.17  0.14

Note: Diff PAS= Difference in penetration aspiration scale score; Diff Suck Time= Difference in suck times reported in seconds; Diff Sucks/Swallow= Difference in number of sucks per swallow; Diff OTT= Difference in oral transit time reported in seconds; Diff PTT= Difference in pharyngeal transit time reported in seconds; Diff LC= Difference in time to laryngeal closure reported in seconds;
Note Cont: Diff LCD= Difference in duration of laryngeal closure reported in seconds; Diff PCD= Difference in pharyngeal constriction duration reported in seconds; Diff CPS= Difference in cricopharyngeal sphincter opening reported in seconds.

Discussion

This study revealed a predictable pattern of median differences between five of the eight evaluated physiologic measures of swallow function noted between swallows with varying degrees of airway invasion. Four of the physiologic measures of swallowing function (time spent sucking, duration of laryngeal closure, pharyngeal constriction duration, and duration of cricopharyngeal sphincter opening) were all higher values for swallows that resulted in more airway invasion (Swallow 2). In contrast, the measure of pharyngeal transit time was consistently longer during swallows of less airway invasion (Swallow 1). Although these five measures show a predictable pattern of median change when comparing swallows with varying degrees of airway compromise, they failed to reach the level of statistical significance. This leaves the clinician and the researcher to consider other possible explanations for the variation in degree of airway invasion that is often seen during continuous suckling bursts.

Considering that the participants in this study spent a greater amount of time sucking during the oral phase of swallows that resulted in greater degrees of airway invasion, it is plausible to consider that participants might be extracting greater volumes of liquid during swallows that result in greater degrees of airway compromise. Previous research has established that healthy infants without dysphagia demonstrate a decrease in the volume of liquid extracted during a suck as a function of maturation, and they have established normative values for volume per suck based on age [21]. The average age of participants in this study was 6.4 weeks. According to established normative values, healthy infants without dysphagia extract an average of 0.40 mL of liquid per suck [21]. In swallows that result in greater degree of airway compromise, it is possible that the volume of liquid extracted per suck may exceed the physical limitations of the infant’s oropharyngeal boundaries [22] and/or exceed their maturational ability to successfully manage the sucking, swallowing, and breathing coordination required for boluses that exceed average volumes per suck [23].

Safe swallowing depends on the coordination of sucking, swallowing, and breathing. When infants present with dysphagia that includes episodes of airway invasion, it is often caused by incoordination of sucking, swallowing, and breathing [8,24,25]. Respiratory cessation during swallowing, commonly known as deglutition apnea, is necessary for safe swallowing. Previous research has shown that infants demonstrate this necessary pause in respiration during swallowing (deglutition apnea) during nutritive and non-nutritive sucking beginning at birth [26-29]. The timing of the swallow and the deglutition apnea during the respiratory cycle is variable and undergoes maturational shifts during the first year of life in healthy infants [30]. Many common pediatric conditions like the ones that the participants in this study demonstrated (prematurity, gastrointestinal disorders, pulmonary conditions, cardiac disorders, and neurologic disorders) can negatively impact the coordination of sucking, swallowing, and breathing and are known to be correlated with pediatric dysphagia [8]. Respiratory measures are not routinely collected during the clinical, infant VFSS and due to the retrospective nature of this research, could not be analyzed. The physiologic measures of swallowing function reported in this study and available for use in the clinical setting were not sensitive for detecting the timing of the swallow and the deglutition apnea during the respiratory cycle. The addition of this information might help explain the variability in degree of airway invasion between swallows.

Limitations of this study include a conservative sample size (N=10) and retrospective analysis. The small sample size limited the statistical power of this research project. The authors also acknowledge the exploratory nature of this pilot work. Understanding infant dysphagia is an ever-evolving area of study. Due to advances in...
neonatal and pediatric medicine, infants and children with complex medical conditions are surviving longer, and therefore speech-language pathologists are faced with the challenges of diagnosing and treating pediatric dysphagia more often in their clinical practice.

Previous literature has established the clinical usefulness of the VFSS to evaluate infant swallowing function and has provided the clinician with descriptions of reliable temporal and physiologic measures that can be derived from the VFSS. However, there is no empirical evidence demonstrating that clinicians routinely use any of the previously described temporal and physiologic measures when administering a VFSS for pediatric populations. Use of these reliable temporal and physiologic measures will provide standardized descriptions of infant swallowing function and assist in the differentiation of potential patterns of swallowing function associated with various medical conditions. Differentiation of the cause of airway compromise during the infant swallows will provide for further specificity of recommendations for compensatory strategies and habilitative or rehabilitative interventions.

The current study is a first step towards this next phase in pediatric dysphagia study. This research attempted to apply the previously-established reliable measures that can be derived from VFSS analysis to elucidate a specific change in swallow function that then resulted in increased airway compromise for infants with dysphagia. As a pilot study, this research included a convenience sample of infants with dysphagia resulting from common pediatric medical diagnoses because there is not currently evidence in the research to definitively distinguish different profiles of swallowing function associated with different medical diagnoses for infants and children. Investigations into potential specific profiles of swallowing function correlated to specific medical conditions known to be associated with pediatric dysphagia, such as prematurity, congenital heart disease, gastrointestinal dysfunction, etc., is warranted. Future studies could contribute to diagnosis-specific knowledge of swallowing function by utilizing a more homogenous participant sample.

Utilizing the reliable infant VFSS measures of time spent sucking, oral transit time, number of sucks per swallow, pharyngeal transit time, time to laryngeal closure, pharyngeal constriction duration, and cricopharyngeal opening duration, this study did not find significant differences in infant swallowing function between swallows that resulted in varying degrees of airway compromise. While these measures are commonly used in research and in clinical practice, the clinical usefulness of these measures has not previously been established as a way to differentiate safe and unsafe swallows. Based on the results of this analysis, future research should investigate the influence of volume and respiratory function on variability of airway invasion during swallows. These considerations will further clinicians’ understanding of the causes of variability in airway invasion during the consecutive suck, swallow, breathe cycles and guide the implementation of effective treatment options.

**Conclusion**

In this pilot study, the authors demonstrated predictable patterns of median change for the majority of infant swallow measures collected between swallows of varying degrees of airway compromise. None of the observed changes were found to be statistically significant. However, the results are useful for informing future research to determine quantifiable measures of infant swallowing function that show significant changes for swallows with varying degrees of airway compromise.
Statement of Ethics
This pilot study involved a secondary analysis of an existing dataset of archived infant VFSS. All participants were de-identified and coded for review in both studies to protect privacy. As mentioned in the manuscript, IRB approval was received from The University of Alabama and The University of Tennessee Health Science center prior to study initiation.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Funding Sources
The authors received no financial support for the research, authorship, and/or publication of this article.

Author Contributions
The three authors each contributed substantially to this research as well as the creation of this manuscript. Dr. Memorie Gosa was responsible for the conception and design of the research and securing IRB approval. Sarah Trulove and Bailee Gist made substantial contributions to data collection. All authors contributed to data analysis and interpretation. All authors took part in the drafting, revising, and final approval of the manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the integrity of the work have been appropriately investigated and resolved.

References

[16] Gosa MM. Determining the effective pulse rate for accurate interpretation of airway compromise during infant modified barium swallow studies. Poster presented at 23rd Annual Dysphagia Research Society Meeting; March 12-14, 2015; Chicago, IL.


Oral Retinoic Acid Treatments Modulate Adipose Tissue Development of Neonatal Offspring of Sprague-Dawley Rats Consuming a High Fat Diet

Heleena Haberer¹, Yanqi Zhang¹, Hui Wang², Libo Tan²*¹

Faculty Mentor: Libo Tan

¹Department of Human Nutrition and Hospitality Management, University of Alabama, Tuscaloosa, AL 35487
²Department of Human Nutrition and Hospitality Management, University of Alabama, Tuscaloosa, AL 35487
*Corresponding author

Retinoic acid (RA), the active metabolite of vitamin A, is a key regulator of adipose tissue development in adult models. This study was done to assess the effects of repeated RA treatments on the adipose tissue development of neonatal rats reared by mothers consuming a high-fat diet (HFD).

Five pregnant Sprague-Dawley rats were randomized to either a normal-fat diet (NFD=25% fat) or an HFD (50% fat). On postnatal day 5 (P5) and P8, respectively, n=12 pups in the HFD cohort received an oral RA dose (HFD+RA group). The remaining pups in the HFD cohort (n=12; HFD group) and pups in the NFD cohort (n=12; NFD group) both received canola oil as a placebo. Six hours after dosing on P8, n=4 pups/group were euthanized, and blood, liver, visceral white adipose tissue (WAT), and brown adipose tissue (BAT) were collected. On P11 and P14, the remaining pups in NFD, HFD, and HFD+RA group (n=8/group) received their treatment. Six hours after the administration on P14, n=4 pups/group were euthanized. On P17 and P20, the remaining pups (n=4/group) received their respective treatment and were euthanized six hours afterward on P20. Serum samples from P14 and P20 were analyzed for concentrations of lipids and adipokines.

At P8 and P14, no significant difference in body weight (BW) gain, WAT mass, and BAT mass of pups were noted among groups. At P20, all the three measures were significantly higher (p<0.05) in the HFD group than in the NFD group; the measures were significantly decreased in the HFD+RA group compared to the HFD group. The serum adiponectin and leptin concentrations were both significantly higher in the HFD than in the NFD group; RA treatment significantly reduced the concentrations of both. At P14 and P20, serum triglyceride was significantly higher in pups receiving RA treatment. RA was also found to improve the vitamin A status of neonatal rats by increasing hepatic storage.

In conclusion, repeated RA treatments exerted a regulatory role on the adipose tissue metabolism and development of neonatal offspring of mothers consuming an HFD, as evidenced by reduced BW gain and adipose tissue mass as well as modulation of adipokines.

Introduction

A joint analysis by UNICEF, WHO, and the World Bank indicated the number of overweight children <5 years old is expected to reach a prevalence of 11% by the year 2025 [1]. The upward trend in children who are overweight or obese is seen across high-income, middle-income, and low-income countries [2]. According to the Centers for Disease Control and Prevention (CDC) [5], in 2019 there was an 18.5% prevalence of obesity between the ages of 2 and 19 in the United States. Childhood overweight/obesity is reported to be closely related to maternal obesity. It was estimated that there were 38.9 million overweight and obese pregnant women in 2014 [6]. The peripartum stage is the most determinant of obesity in both the mother and infant [24]. If the mother becomes overweight/obese during this phase of pregnancy, the infant may become obese due to genetic factors or environmental factors of the womb [24].

Retinoic acid (RA), the active metabolite of the essential micronutrient vitamin
A (retinol), has been reported to be a key regulator of adipose tissue development in adult obese models [21]. RA is a hormone that can interact with nuclear transcription factors and regulate gene expression. Previous research reported that the “machinery” required for the molecular action of RA, including retinoic acid receptors (RARs) and retinoid X receptors (RXRs), are all expressed in the adipose tissue [9]. Our previous study indicated that maternal dietary vitamin A supplementation significantly reduced adiposity and improved the metabolic health of neonatal and weaning rat offspring from mothers consuming a high-fat diet (HFD) [19]. However, the effect of direct RA treatment on obesity development in neonates had not been investigated.

Therefore, the objective of the study was to assess the effects of repeated oral RA treatments on the body weight (BW) and adipose tissue development of neonatal rat offspring from dams consuming an HFD. A single oral dose of RA was reported to have a transient effect on the metabolism of neonatal rats, and therefore RA was administered repeatedly.

Materials and Methods
Animal Experiment
The procedure for this experiment was approved by the Institutional Animal Care and Use Committee of the University of Alabama. Five pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, USA) and arrived on their second day of gestation. Rats were housed individually with a 12-hour light/dark cycle with free access to food and water. After a 3-day acclimation, rats were randomized to either a normal-fat diet (NFD=25% fat; n=2) or an HFD (50% fat; n=3) both with a marginal level of vitamin A at 0.35 mg/kg. The diets were purchased from Research Diets, Inc. (New Brunswick, NJ, USA). After delivery, half of the pups delivered by HFD mothers received oral RA treatments, while the other half of the HFD pups and pups of the NFD mothers received canola oil as a placebo. The three groups of pups (n=12/group) were designated as NFD, HFD, and HFD+RA, respectively.

The schematic diagram of the study design is shown in Figure 1. Specifically, on postnatal day 5 (P5) and P8, respectively, HFD+RA pups received an oral RA dose via feeding pipette at 4 µg/g BW. HFD pups and NFD pups both received canola oil at the same dosage. Six hours after the dose administration on P8, n=4 pups/group were euthanized. Blood, liver, visceral white adipose tissue (WAT), and brown adipose tissue (BAT) were collected. On P11 and P14, the remaining pups in each group (n=8/group) received a dose of oil (NFD pups), oil (HFD pups), and RA (HFD+RA pups), respectively. Six hours after the administration on P14, n=4 pups/group were euthanized for tissue collection. On P17 and P20, the remaining pups (n=4/group) received their respective treatment and were euthanized six hours afterward on P20. Pups’ BW and the weights of WAT and BAT were recorded. Pups’ BW gain was calculated as the BW at the euthanization time minus that at P4.
Serum and Tissue Analysis

Serum Lipids and Adipokines
Serum samples from P14 and P20 euthanization were analyzed for concentrations of lipids and adipokines. Serum samples from P8 euthanization were not adequate for the analyses. Concentrations of total cholesterol, triglycerides, HDL-C, and LDL-C were measured directly using a Stanbio Sirus analyzer. Adiponectin concentration was assessed using a Millipore Rat Adiponectin ELISA (Billerica, MA), and leptin was measured using a Millipore Rat Leptin ELISA.

Serum and Liver Vitamin A Concentration
The total retinol concentration in serum and liver was analyzed by ultra high-performance liquid chromatography (UPLC) (Acquity UPLC System; Waters, Milford, MA, USA) following our previous method [26]. Briefly, 100 µL of serum sample was added to 1.9 mL of ethanol and incubated for one hour. Saponification was achieved by adding 100 µL of potassium hydroxide and 100 µL of 20% pyrogallol to samples followed by incubation in a 55°C water bath for 30 minutes. After cooling down, 4 mL of hexane and 2 mL of dd H2O were added. The sample was then centrifuged for 15 minutes, and the upper surface was collected and dried under nitrogen. The dried sample was rinsed by hexane and reconstituted with 100 µL of acetonitrile:methanol (85:15, v/v). Possible precipitation was removed by centrifugation. The final sample was injected onto the Waters Acquity UPLC HSS T3 (1.8 µm, 2.1 mm × 100 mm) column (Waters, Milford, MA, USA) for analysis. Retinyl acetate (Sigma-Aldrich, St. Louis, MO, USA) was used as the internal standard.

Statistical Analysis
Data are reported as means ± standard error of the mean (SEM). Differences among groups, p<0.05, were determined by using one-
way ANOVA followed by Bonferroni post-test in Prism software (GraphPad, San Diego, CA).

Results

Body Weight and Adiposity

As shown in Figure 2, at P8 and P14, no significant difference in the BW gain, WAT mass, and BAT mass of pups was noted among groups. At P20, all three measurements were significantly higher in the HFD group than in the NFD group (BW gain: 59.05 ± 2.57 g vs. 49.63 ± 1.54 g, P<0.001; WAT mass: 1.03 ± 0.10 g vs. 0.59 ± 0.06 g, P<0.0001; BAT mass: 0.51 ± 0.04 g vs. 0.33 ± 0.02 g, P<0.0001), confirming that maternal HFD consumption during gestation and lactation could result in a significantly higher BW gain and excessive adiposity in the neonates. At P20, both the BW gain and the WAT mass were significantly decreased in the HFD+RA group compared to the HFD group (BW gain: 50.15 ± 2.42 g vs. 59.05 ± 2.57 g, P<0.01; WAT mass: 0.89 ± 0.04 g vs. 1.03 ± 0.10 g, P<0.05), showing the effects of RA treatment on slowing the BW gain and reducing the adiposity. There was no significant difference noted in the BAT mass between the HFD+RA and the HFD group (0.46 ± 0.02 g vs. 0.51 ± 0.04 g, P>0.05).

Serum Adiponectin and Leptin

At P20, serum leptin and adiponectin concentrations (Figure 3 A&B) were both significantly higher in the HFD than the NFD group (leptin: 28.64 ± 1.15 ng/mL vs. 12.14 ± 1.99 ng/mL, P<0.0001; adiponectin: 19.11 ± 2.24 µg/mL vs. 12.54 ± 0.62 µg/mL, P<0.05). Comparing the HFD+RA and the HFD group indicated that RA treatment significantly reduced the concentrations of both (leptin: 20.18 ± 2.70 ng/mL vs. 28.64 ± 1.15 ng/mL, P<0.01; adiponectin: 15.67 ± 2.59 µg/mL vs. 19.11 ± 2.24 µg/mL, P<0.05). The pattern of changes in serum leptin was also observed at P14.

Serum Lipids

At P20, serum triglycerides concentration (Figure 3C) was found to be significantly higher in the HFD group than the NFD group (400.75 ± 57.56 mg/dL vs. 206 ± 24.46 mg/dL, P<0.05). The concentration was even higher in the HFD+RA group as compared to the HFD group (1104 ± 297 mg/dL vs. 400.75 ± 57.56 mg/dL, P<0.05). A similar trend was noted at P14, but the difference between the HFD+RA and the HFD group did not reach statistical significance. There was no significant difference in serum’s total cholesterol, HDL-C, and LDL-C observed among groups.

Figure 2: The body weight gain (A), visceral white adipose tissue mass (B), and brown adipose tissue mass (C) of rat pups at postnatal day 8, postnatal day 14, and postnatal day 20. The bodyweight gain was calculated as the bodyweight at the given time point minus that at postnatal day 4. Bars show means ± SEM, n=4 per group. Different letters indicate statistically significant differences, a”>b”>c”, P<0.05.
Figure 3: Serum leptin (A), adiponectin (B), and triglycerides (C) concentrations of rat pups at postnatal day 14 and postnatal day 20. Bars show means ± SEM, n=4 per group. Different letters indicate statistically significant differences, a>b, a’>b’>c’, P<0.05.

Serum and Liver Vitamin A

The total retinol concentrations in pups’ serum and liver are shown in Figure 4. Comparing between the NFD and the HFD group, no significant difference was noted, except at P20 where the HFD group exhibited a significantly lower liver total retinol (0.025 ± 0.002 µmol/g vs. 0.040 ± 0.002 µmol/g, P<0.05). At P8, the serum total retinol was significantly lower in the HFD+RA than the HFD group (0.58 ± 0.07 µmol/L vs. 0.90 ± 0.12 µmol/L, P<0.05), while the liver total retinol was significantly higher in the HFD+RA group (0.060 ± 0.005 µmol/g vs. 0.050 ± 0.004 µmol/g, P<0.05). At P20, the HFD+RA group also possessed a significantly lower serum total retinol but a significantly higher liver total retinol concentration compared to the HFD group (serum retinol: 0.380 ± 0.06 µmol/L vs. 0.730 ± 0.08 µmol/L, P<0.05; liver retinol: 0.034 ± 0.003 µmol/g vs. 0.025 ± 0.002 µmol/g, P<0.05). There was no significant difference in serum nor liver total retinol among groups at P14.

Figure 4: Concentrations of total retinol in serum (A) and liver (B) of rat pups at postnatal day 8, postnatal day 14, and postnatal day 20. Bars show means ± SEM, n=4 per group. Different letters indicate statistically significant differences, a>b, a’>b’>c’, P<0.05.

Discussion

To the authors’ knowledge, the present study was the first to investigate the effects of oral RA treatment on the adipose tissue development and metabolism of neonatal rats in an obesogenic environment. It was found that repeated RA treatments during the suckling
period resulted in a beneficial regulatory role in obesity development.

Maternal HFD consumption during gestation and lactation (up to 20 days) was shown to dramatically increase the adiposity and the BW gain of the neonates, which is consistent with previous findings from others [4,10] and from this study [19]. The negative effects of maternal obesity or excessive gestational weight gain on the metabolic health of the offspring has been well established. In the current study, it was found that repeated RA treatments given orally to the pups every three days from P5 to P20 exerted a significant effect on reducing the BW gain and the WAT mass. The findings are consistent with our previous study, which showed that maternal dietary vitamin A supplementation significantly reduced the BW and the adiposity of suckling and weanling pups of dams consuming an HFD. Although maternal dietary supplementation would pose less risk to the offspring, it can only be utilized in the lactational period. In contrast, direct administration of the treatment to the offspring would allow for a potentially long-lasting effect.

Previous studies in adult models indicated that RA inhibited adipogenesis [1,22] but stimulated angiogenesis and apoptosis [11] of the WAT, which may explain why the treatment can reduce the WAT mass. In addition, RA was found to increase the adaptive thermogenesis of BAT [8] and to induce the browning of WAT [23] or remodeling of WAT to BAT [14]. BAT is the site for adaptive thermogenesis and is prominent in newborns. In humans, it is gradually lost with age, but the body may still contain beige adipocytes that can be potentially reactivated. Therefore, BAT retains the capacity to play a significant role in energy metabolism and is a primary target in obesity prevention and treatment [18]. Our previous study applying maternal dietary vitamin A supplementation showed that maternal consumption of HFD significantly reduced BAT mass while vitamin A supplementation restored the mass, which may potentially contribute to a higher level of adaptive thermogenesis and help with weight control. However, in the present study, the maternal HFD consumption increased the BAT mass in the neonates, and the oral RA treatment did not exert any effect on the mass. The discrepancy between the present and the previous study needs further investigation. However, the finding from the present study that RA treatment significantly reduced the neonatal WAT mass but did not exert the reducing effect on the BAT is encouraging.

Leptin and adiponectin are two adipokines that are primarily produced by the WAT and correlate with obesity and metabolic health. Leptin can reduce fat storage in adipocytes by inhibiting hunger. Adiponectin plays a role in regulating glucose homeostasis and fatty acid breakdown. In the present study, maternal HFD consumption was found to significantly increase the serum leptin and adiponectin concentrations in neonatal rats, and RA treatment significantly reduced both concentrations. The changes may simply be the result of increased WAT mass in HFD pups and reduced tissue mass by the RA treatment. The findings on serum leptin are consistent with our previous study, in which maternal dietary vitamin A supplementation was also found to decrease the enhanced serum leptin concentration in HFD pups [19]. Previous studies in adult rodent models also showed that chronic dietary vitamin A supplementation reduced serum leptin as well as leptin gene expression in WAT [8,13]. Acute RA treatment was shown to downregulate the gene expression of both leptin and adiponectin in WAT in adult rats [25] as well as suppress leptin gene expression in BAT [12]. The potential physiological benefits or consequences of RA’s inhibitory effects on leptin and adiponectin production will need further exploration.

Serum lipid profile was determined in the current study. It was noted that the concentration of serum triglycerides was significantly higher in HFD pups compared to NFD pups and was further enhanced in HFD+RA pups. The effect exerted by RA was surprising, considering that RA reduced the mass of WAT where triglycerides are stored. There were previous case studies reporting that serum triglyceride concentration was increased in patients receiving isotretinoin (13-cis-RA) as acne treatment [7] or following a high dose vitamin A treatment to patients with pityriasis
rubra pilaris [15]. Although the mechanism is unknown, it is plausible that vitamin A or RA promoted lipolysis in WAT, as shown previously [3]. More triglycerides may resynthesize from fatty acids and glycerol in the liver, which are packed into very low-density lipoproteins and secreted into the circulation, resulting in a higher serum concentration.

Lastly, the serum and liver vitamin A concentrations of the neonatal rats were observed to see how maternal obesity and RA treatment may have affected vitamin A status. The liver is the primary storage organ for vitamin A. At P8 and P14, no significant difference in serum or liver total retinol concentration was noted between the NFD and the HFD group. However, at P20, maternal HFD consumption significantly reduced the neonatal liver vitamin A concentration, bringing it from a marginal to a deficient status, which is consistent with previous research affirming that the hepatic vitamin A concentration of HFD-fed adult Wistar rats was ~50% that of the controls [17]. Similar findings were also noted in obese mice [20]. At P8 and P20, the comparison between the HFD and the HFD+RA group indicated that RA treatment promoted the uptake and/or storage of vitamin A in the liver, as evidenced by a significantly decreased serum total retinol but an increased liver total retinol concentration. Previous research showed that acute treatment of RA significantly increased the hepatic gene expression of lecithin retinol acyltransferase (LRAT), the enzyme that is responsible for the esterification/storage of vitamin A [16]. Our study was the first to report that the compromised hepatic vitamin A storage in HFD pups could be improved by RA treatment. The total retinol concentration in WAT and BAT was also assessed, but no significant difference was noted among groups. It is plausible that any potentially increased vitamin A uptake by the adipose tissue in the HFD+RA group was offset by the active utilization of vitamin A in the tissue.

Conclusion

Using a maternal-neonatal Sprague-Dawley rat model, it was found that maternal HFD consumption during gestation and lactation posed a significantly negative impact on the body weight, adipose tissue development, and vitamin A status of the neonatal offspring. Repeated oral RA treatments during the suckling period significantly reduced BW gain and WAT mass, modulated adipokine levels, and improved the vitamin A status of the neonates. This study showed the potential that RA holds to improve the outcome of childhood obesity for infants who are predisposed to obesity. Further analysis at the molecular level will be done to fully understand how RA regulates the metabolism and the development of adipose tissue in neonates. Pre-clinical studies with a longer duration and multiple RA doses are also needed to elucidate the long-term effects of RA and to determine the optimal dose.

Declaration of competing interest

The authors confirm that they have no conflict of interest to declare for this publication.

Acknowledgments

This project is funded by NIH R01 HD066982 Sub-award to LT.

References

Interview with Dr. John Higginbotham

Dr. John C. Higginbotham, PhD, MPH
Senior Associate Vice President and Chief Operations Officer for Research & Economic Development; Associate Dean for Research and Health Policy, CCHS; Director, Institute for Rural Health Research; Professor and Chair, Community and Rural Medicine

JOSHUA Staff (JS): What led to your interest in epidemiology?

JCH: I had never heard of epidemiology until my senior year of college here at UA. We had a guest speaker in one of my courses, he was an epidemiologist. I was so intrigued by what he said that I began to investigate, and the rest is history as they say.

JS: Can you share the personal and professional path that you’ve taken to achieve this career and then work at the University of Alabama?

JCH: After graduating from UA, I went to the University of Alabama in Birmingham, School of Public Health and obtained my master’s degree. From there, I did a preceptorship in Lincoln/Lancaster County Nebraska Health Department, and then went to the University of Texas Medical Branch (UTMB) and got my PhD in Preventive Medicine and Community Health, with a specialty in Epidemiology. I did additional course work and training in Epidemiology at the University of Minnesota and the University of Michigan. I was first a faculty member at UTMB, then accepted a position at the University of Mississippi Medical Center, Department of Epidemiology. In 1999 I joined the UA College of Community Health Sciences, Student Life, Academic Affairs, Financial Affairs, Office of Information Technology, Strategic Communications, Student Health, University Medical Center, Public Safety, Facilities, Transportation, Food Service, Environmental Health and Safety, faculty, staff, and students, and not the least of which are those women and men who make up the COVID-19 Support Program. These people, working together, have made a tremendous difference.

JOSHUA Staff (JS): What led to your interest in epidemiology?
JS: What has been your most exciting advancement in research during your time at the University of Alabama?

JCH: Being a site Principal Investigator for the All of Us Study is probably the most exciting study of which I have ever been a part. This NIH precision medicine study seeks to enroll more than 1 million people from very diverse backgrounds and is working to improve health care through research. Unlike research studies that focus on one disease or group of people, All of Us is sequencing the DNA of every participant and collecting other data that will inform thousands of studies on a variety of health conditions. This creates more opportunities to: know the risk factors for certain diseases; figure out which treatments work best for people of different backgrounds; and inform participants about their individual genetic risk factors. This study will change how we do medicine.

JS: What does a typical day currently look like? How does this compare to your work and life before the pandemic?

JCH: First off, there has been no typical day. However, I have spent a lot of time in meetings, answering phone calls and emails, and working with others trying to problem solve. I look at lots of data and I read lots of reports and, on occasion, when I could, I rolled up my sleeves and helped where I could, in rural areas, at Coleman Coliseum, and several other places.

JS: What was your initial reaction to COVID-19 and how did that change once you were appointed as lead of the COVID-19 response at the Capstone?

JCH: There is a line from a movie that I really like, and it expresses my thoughts at the time, “Its 1918 all over again,” of course referring to the 1918 flu which killed an estimated 100 million people worldwide, COVID-19, as of June 1, 2021 has killed an estimated 3.5 million people worldwide. While COVID-19 has caused a lot of deaths and it has decimated families, it is not the 1918 flu all over again.

JS: How has your background and past research benefited the University of Alabama’s response to COVID-19?

JCH: I believe that my experience in the Lincoln/Lancaster County Nebraska Health Department, my collaborative projects with the Mississippi State Department of Health, and with the Alabama Department of Public Health, as well as my experience with large data collection projects, like the All of Us study and others, gave me the training, knowledge, skills, and experience to work collaboratively with the numerous and various groups necessary to see that our COVID-19 Response is targeted, resilient, and adaptable.

JS: You have done research on racial and ethnic disparities related to health issues. In what ways have these disparities presented themselves during the pandemic?

JCH: Unfortunately, like most health and healthcare issues, COVID-19 has again spotlighted inequalities among both racial and ethnic groups, as well as those living in rural and underserved areas.

JS: How has COVID-19 impacted rural communities, and what obstacles lie in the way of getting vaccines to rural communities?

JCH: When you have seen one rural community, you have seen one rural community. The impact has been different everywhere you turn, many have had problems testing for the virus, others have not. Some rural communities have had difficulties finding places to store the vaccine at appropriate temperatures. Some rural areas were fortunate enough to have the National Guard to come and help vaccinate. Even though some “Mom and Pop” pharmacies as well as chains stores such as Walmart and CVS are now providing vaccinations along with health departments, hospitals, and physician offices, there are still places where vaccines are difficult to find or are difficult to get to because of poor transportation. Regardless of the availability, there is still a hesitancy by some for getting vaccinated.
JS: Has the current pandemic changed any of your thoughts regarding epidemiology in general, and if so, how?

JCH: On more than one occasion when someone asked me what I did, and I responded that I was an epidemiologist, they would say something to the effect of “Oh, you’re a skin doctor.” Now, people seem to have a better idea of what I do.

JS: What advice would you give to prospective students interested in public health?

JCH: Public Health is one of the few professions where you can follow the same topic for a career, e.g., breast cancer epidemiology, or have something different nearly every day and everything in between. To prepare for such a career, study a little of a lot of things. For example, as an undergraduate you might focus on Psychology, Biology and English like I did but throw in some chemistry, an art class, computer science, acting, engineering, or a business class. The best Public Health folks, in my opinion, are renaissance thinkers, while they are very knowledgeable in a specific area, they also know a little about a lot of things which helps them converse with others and think through the issues at hand.

This interview was conducted by Arman Singh, Grace Beauchamp, Amy Pender, Jacqueline Jessop, and Nick Hayes, Assistant Editors on the JOSHUA staff.
Interview with Dr. Russell J. Mumper

Russell J. Mumper, PhD
Vice President for Research and Economic Development

JOSHUA Staff (JS): Regarding your background, what eventually led you to become Vice President for Research and Economic Development at The University of Alabama? Where are you from, where did you go to college, and what did you do prior to taking on this role?

Dr. Mumper (RM): I grew up near Milwaukee, Wisconsin, then my family moved to Kentucky where I completed high school. I attended the University of Kentucky and graduated with two degrees, a B.A. in chemistry and a Ph.D. in pharmaceutical sciences. After I completed a postdoctoral fellowship, I worked in the pharmaceutical and biotech industries for eight years before joining the University of Kentucky in 1999 as a faculty member in the College of Pharmacy. I moved to UNC Chapel Hill in 2007 and was the founding director of the Center for Nanotechnology in Drug Delivery. While I was a faculty member at UK and UNC, I co-founded five companies. I gradually became more interested in administration and especially university-wide administration. I moved to the University of Georgia in 2014 as Vice Provost and then to The University of Alabama in 2019. I feel the Vice President for Research & Economic Development position is an excellent match for my skill set, experiences, and interests. UA is a great place to have this role, as the responsibilities for research and economic development are combined, and UA has great potential to have national impact in several areas of research and creative activities.

JS: What led you to transition from working in an industry setting to working in academia?

RM: This is a difficult question to answer succinctly because there are a few key professional and personal factors that led to such a big decision. On the personal side, our family was getting larger and my wife and I felt the call to be closer to our families in Kentucky, especially after living in three other states. On the professional side, although I loved my time in industry, I felt a growing desire to pursue my own research interests and teach and mentor students.

JS: With regard to your research, what do you consider to be some of your most exciting and impactful advancements over the course of your career?

RM: My perspective on what I consider my most impactful achievements has changed over time. My research has led to highly cited papers, patents, and start-up companies primarily in the area of advanced drug delivery and nanotechnology. Many of the patents have been licensed or acquired by companies and are supporting late state products in human clinical trials. All of this work has been very exciting and by many different metrics has been impactful. However, as I look back now, there is no doubt that my greatest joy and impact are the students I have mentored and trained in research who have gone on to do great things. My former students are now professors at comprehensive research universities and...
directors at pharmaceutical and biotechnology companies. It is difficult to express the sense of fulfilment that their career successes mean to me.

**JS:** What is your vision for the future of research at our institution? Do you have any long-term goals or plans?

**RM:** The research enterprise at UA is thriving like few other research universities in the country. This growth is attributed to successful investments in infrastructure and world-class faculty; the establishment of signature research areas; and the growing number of partnerships with industry and with state and federal agencies. In June 2019, the Office for Research & Economic Development launched a five-year strategic plan for UA research with the overarching goals of doubling the research enterprise in five years and becoming one of the premier comprehensive research institutions in the southeastern United States. However, more important than these goals, is providing differentiating opportunities for our students and to have societal impact, the essence of a public ‘flagship’ university.

**JS:** We noticed that you have written about the benefits of a flipped classroom. What is your personal philosophy for teaching?

**RM:** In 2014, I led a three-year initiative on the flipped classroom, which studied student performance, engagement, and perceived value of a flipped classroom model versus the traditional lecture model. A strong motivation for the initiative was to march up Bloom’s Taxonomy of learning, focus student learning on higher-order thinking, problem solving, and critical thinking, to, “better prepare students for success in today’s global economy.” In essence, I produced short, content rich learning materials the students referred to before class, then the entire class time was spent reinforcing and discussing those concepts and applying them to real-world problems in pharmaceutical dosage forms. The three-year study, published in Academic Medicine, showed that 91% of students strongly agreed or agreed that the overall course format of the flipped classroom greatly enhanced their learning. Preference for the traditional lecture format decreased from 109 students (72.7%) in the precourse survey to 23 students (15.4%) in the postcourse survey, while preference for the flipped classroom format increased from 41 students (27.3%) to 126 students (84.6%).

**JS:** Finally, what advice would you give to students interested in getting involved with undergraduate research here at The University of Alabama?

**RM:** Another major factor that motivated me to pursue the position of Vice President for Research & Economic Development at UA is that the Office for Undergraduate Research is within my purview. Undergraduate research is personal to me. When I was an undergraduate chemistry student at the University of Kentucky, I was required to do three semesters of independent research. By complete chance, a few conversations led me to the College of Pharmacy where I met a professor interested in hosting me and being my mentor. It was chance because I had never stepped foot into the College and was naïve about the type of research they performed. The short story is that within a month of beginning my independent research, I knew exactly what I wanted to do with my future. The experience changed everything for me. I give students the advice to be open to possibility and to not be shy about reaching out across campus and approaching faculty.

*This interview was conducted by Jenna Bennett, Executive Editor on the JOSHUA staff.*
Interview with Dr. Sharlene Newman

Sharlene Newman, PhD
Executive Director for the Alabama Life Research Institute; Professor, Department of Psychology

JOSHUA Staff (JS): Regarding your background, what eventually led you to become the executive director of the Alabama Life Research Institute (ALRI) at The University of Alabama? Where are you from, where did you go to college, and what did you do prior to taking on this role?

Dr. Sharlene Newman (SN): I am from Abbeville, Alabama (southeast Alabama). I got my bachelor of engineering in electrical engineering and math from Vanderbilt University and my master’s and PhD in biomedical engineering from UAB. While in grad school, I became really interested in neuroscience and did postdoctoral training at Carnegie Mellon in the psychology department where I became a cognitive neuroscientist. It is a convoluted route to cognitive neuroscience, but you also have to understand that the field is relatively new. Cognitive neuroimaging is also relatively new. Plus, I grew up in a small town, and because I did well in math, everyone told me I should be an engineer so that’s what I did. I didn’t find neuroscience until grad school.

After my postdoc, I became a professor at Indiana University in Bloomington. I was hired, along with one other junior faculty member, to help start their neuroimaging program. It was a great experience as we were able to help plan the new MRI imaging facility. I later became the director of that facility. I rose through the tenure ranks at IU and took on administrative roles in addition to the director of the imaging facility. I was an associate vice provost for undergraduate education where I focused on STEM retention, experiential learning including undergraduate research and service learning, women in STEM, and prestigious awards (e.g., Rhodes, Fulbright, etc.) I also oversaw the undergraduate research journal. I was also the director of the graduate program in neuroscience and am proud to say that I helped to eliminate the GRE requirement and increase the number of underrepresented student applicants and students.

When I learned about the position as executive director of the Alabama Life Research Institute, I was drawn to it for a number of reasons. It seemed like a great fit for me because of its interdisciplinary research focus. My entire career is interdisciplinary, not only my education/training but my research. I have collaborated with researchers from multiple disciplines as you can see from my CV. I also saw a lot of potential here at UA. The university recently became a research-intensive university, which suggested that it was ready to grow in this area. I wanted to be a part of that growth in my home state. Finally, ALRI has a focus on research that can affect the lives of people in Alabama, particularly rural Alabama. This was important to me as someone who was born and raised in rural Alabama. So it really felt like a no-brainer to me.

JS: Can you explain what life research is and some of the work that is currently being done at ALRI? What are your goals for the institute and your vision for the future?

SN: This is a hard question in some ways. It is actually a question I asked when I came to interview. Life covers a lot of territory. And the mission statement includes the phrase from the “molecular to the environmental.” There is a lot in between those two. It was clear to me that UA faculty, the people who would actually be doing the research, needed to define what life research means for ALRI. I spent my first 9 months to a year on campus learning from the faculty where the expertise lies and where there was great potential for growth. Honestly, I know I have not been able to reach all parts of the campus in
that time. Plus, covid happened making it even more difficult to do so.

We were, however, able to create a strategic plan for the ALRI and identify five research themes that we will focus on. I do want to emphasize that while we are focused on these themes, that doesn’t mean that the institute will not engage faculty who work outside of them. The themes are:

1. Rural health and health disparities
2. Neuroscience
3. Convergent biosciences and medicine
4. Implementation science
5. Environmental science and health

JS: Cross-disciplinary collaboration seems to be a defining aspect of your career and approach to research. Can you share why the collaboration of researchers from different fields is critical for making scientific advancements and why such an approach is especially important for life science research?

SN: ALRI is focused on addressing big systemic, often intractable, problems. These problems are big and difficult to solve because they are multifaceted and therefore require experts from different areas working together to solve them.

JS: Regarding your research, what do you consider to be some of your most exciting and impactful advancements over the course of your career? Are there any topics you have not yet explored but would like to in the future?

SN: I have seen neuroimaging change a lot over my career. I think it is also important to remember that my training is in engineering so that colors a bit how I see brain functioning. In the early days, I would say there was a lot of focus on labeling specific functions to brain areas. Cognitive neuroscience was heavily influenced by the deficit model where the function of a brain region was determined by the cognitive deficit that resulted from damage to the region. The most popular example of that is Broca’s area where Paul Broca found that people who had a stroke that damaged the region had difficulty with speech production and some language functions. Over time we have slowly moved away from this and are focused more on what is happening in “normal” brains and the understanding that the brain is a complex, dynamic network with lots of feedback and feedforward loops. I hope what is next is a focus on individuals and the effect of environmental exposures on brain structure and function. That is the direction I would like to explore in the future, what is responsible for individual differences in brain.

Warning, I’m going to get on my soapbox for a second. I would like to help de-stigmatize mental health. There is this belief that mental health is some abstract thing that is different from physical health. But it is linked to brain, which is physical. The medications work on brain. If we do not stigmatize someone with cardiovascular disease, then why do we stigmatize someone with ADHD or autism or depression? I would like to see neuroscientists being activists in this space to make people more comfortable seeking help for mental illness, including addiction.

JS: We understand you have extensive experience and expertise in brain imaging technology such as MRI. What are some unique applications of this technology we might expect to see in the future? How will you personally utilize such methods at ALRI?

SN: I am really excited about the new MRI research facility we are creating here at UA. It will be a big part of what ALRI is planning for the neuroscience theme. I started my MRI research in grad school at UAB back in the dark ages of functional MRI. I love the technology because of its versatility. You can obtain exquisite anatomical images, including using water diffusion to examine properties of white matter, examine brain functioning during task performance or at rest, brain chemistry using spectroscopy, perfusion, and more. Also, while I have focused on brain, the scanner is a whole-body scanner, so we will also be able to do cardiac, musculoskeletal, any body part imaging.
I am looking forward to the MRI scanner coming to campus and am so ready to collaborate with faculty and work with students. My research has focused on cognition, including language processing and second language learning, and problem-solving, including mathematical problem-solving. I have also worked with clinical populations, including substance abuse (mostly cannabis), concussion, and dementia. I am already developing collaborations in the dementia and concussion space and already have a collaborator in educational neuroscience, Dr. Firat Soylu. We are also planning some collaborations with UAB.

**JS:** Finally, what advice would you give to undergraduate students interested in going into this field?

**SN:** My advice to undergraduates is always to get involved in some form of experiential learning. A lot of learning happens outside of the classroom. If you can work with a faculty member or graduate student to learn more about what research is then do so. Apply for summer research experiences on other campuses. Get a summer internship at a company. Do a service learning project. You can’t really know what you like or want to do until you try it out. That means you should start early in your undergraduate career because you may need to try a few things out before you find what fits you.

*This interview was conducted by Jenna Bennett, Executive Editor on the JOSHUA staff.*
JOSHUA was made possible by the following organizations:

University of Alabama Office for Academic Affairs

University of Alabama Department of Biological Sciences

Tri-Beta National Honor Society Kappa Beta Chapter

Howard Hughes Medical Institute

Randall Research Scholars Program

And by the 2020-2021 JOSHUA Editing Staff

2022 Submission Guidelines

We accept articles from current undergraduate students at accredited universities. If you are a graduate student or recent alumnus of UA, we will consider your article if the majority of your work was conducted while you were an undergraduate at UA. Undergraduate students from other institutions may submit; however, priority will be given to those who conducted their research at UA.

1. Your name, e-mail address, and phone number must be included.
2. Your submission must relate to science or health.
3. Your work must be sponsored by a faculty member.
4. The length of your submission must be between 2000 and 4500 words. We will accept longer submissions if the author can limit the submission to the required length for the publication, and any extra material is able to be published online.
5. Figures, charts, and graphs are allowed but not required. (Note: The color will be mostly black and white.)
6. Your paper must contain an abstract.
7. Your citations must follow the guidelines listed on our website at: https://joshua.ua.edu/submissions-and-guidelines.html
8. The deadline for submission is February 28, 2022.
9. E-mail submissions to joshua.alabama@gmail.com