Examining Phospholipids after Oxidative Stress in the Membrane of a *C. elegans* Alzheimer's Model and Culture of Safety Analysis in New York Hospitals

A Major Qualifying Project Report:
Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

Liza Hote
Biochemistry and Professional Writing

Submitted: April 28th, 2022

Approved by:
Carissa Olsen, PhD
Chemistry & Biochemistry

Yunus Telliel, PhD
Humanities & Arts

This report represents the work a WPI undergraduate student submitted to the faculty as evidence of completion of a degree requirement. WPI routinely publishes these reports on the web without editorial or peer review. For more information about the projects program at WPI, please see http://www.wpi.edu/academics/ugradstudies/project-learning.html

Acknowledgements

I would like to thank the following people for their support throughout this MQP:

- Dr. Carissa Olsen, for her constant feedback, patience, and attention to detail.
- Dr. Brenton Faber for his creativity and guidance.
- Dr. Yunus Telliel for his patience and advice.
- Andre Vieira for his positivity in the lab and teaching me how to use the GC-MS.
- Mark Xatse for teaching me how to be a scientist and how to use the HPLC-MS.
- Connor Norton for collecting replicates for me and teaching me how to make media.
- My professional writing partner Silvana Reid for her problem-solving abilities and positive attitude.

Forward

This Major Qualifying Project consists of two parts. The first was my biochemistry research project where phospholipids in the membrane of *C. elegans* were analyzed after oxidative stress. The next piece, working in tandem with my partner Silvana Reid, quantified the culture of safety in New York hospitals. The two projects were related by an analysis of the impact of the culture of safety on WPI biomedical laboratories.

Table of Contents

Part 1: Examining Phospholipids after Oxidative Stress in the Membrane of a C. elegans Alzheimer's Model

Author:
Liza Hote, Biochemistry and Professional Writing

Advisor:
Dr. Carissa Olsen, Ph.D., Department of Chemistry and Biochemistry

1.0 Abstract

Recent studies have shown a decrease in plasmalogens, a unique class of ether-linked phospholipids, in the Alzheimer's brain prior to cognitive decline. Thus, plasmalogens have potential as a biomarker for early Alzheimer's detection or as a therapy, but the connection between plasmalogens and Alzheimer's is not completely understood. Plasmalogens most putative function for Alzheimer's is as sacrificial antioxidants that would limit oxidative damage in a cell. To further study these lipids, a *fard-1* mutant in *C. elegans* was used as these animals are incapable of making plasmalogens. Specifically, we investigated the role of plasmalogens in responding to oxidative stress caused by elevated dietary sugar (glucose and galactose). Wildtype (N2) animals and *fard-1* mutants were fed a diet with or without sugar and their lipids were extracted then analyzed by gas chromatography/mass spectrometry for membrane fatty acids and HPLC with an orbitrap mass spectrometry for intact phospholipid species. On a regular diet, *fard-1* exhibited a decrease in polyunsaturated fats, and increase in moderately unsaturated fats. Surprisingly, no changes were identified in *fard*-1's response to additional oxidative stress, glucose and galactose. Taken together, future work should further elucidate the role of plasmalogens in response to oxidative stress. This may provide evidence that plasmalogens can be harnessed as a therapy or biomarker.

2.0 Introduction

Alzheimer's disease (AD) is the most common form of dementia and cause of decline in cognitive ability. AD is a neurodegenerative disease that usually affects people over the age of 65. It significantly interferes with social and occupational functioning, specifically affecting language, memory, comprehension, attention, judgment and reasoning. There is currently no cure for AD, thus it is critical to understand disease pathology to inform therapies or future biomarkers (Shaheen, 2022).

Recent studies have demonstrated that the decrease in plasmalogens is correlated with the severity of AD. Plasmalogens are a unique class of membrane glycerophospholipids containing a fatty alcohol with a vinyl-ether bond at the *sn*-1 position and enriched in polyunsaturated fatty acids (Braverman & Moser 2012). Plasmalogens are thought to act as "scavengers," or sacrificial antioxidants meaning they get preferentially oxidized but do not increase the oxidative burden in the cell. This contrasts with glycerophospholipids containing polyunsaturated fats (PUFAs) that get oxidized creating lipid peroxides that are harmful to the cell.

It is currently not understood whether the lack of plasmalogens is the cause or a downstream effect of AD. There are a few hypotheses describing potential roles of plasmalogens in AD etiology. Reduced plasmalogens might further enhance ongoing oxidative damage in AD, as well as alter membrane structural integrity and protein function which ultimately facilitates plaque and tangle production. Alternatively, the decrease in plasmalogens may be related to decreased synthesis secondary to general loss of peroxisome functions in the AD brain. Regardless, understanding the role of plasmalogens in AD has potential as a new therapy, or a biomarker for AD. In a 2017 study, Fujino et al, demonstrated that oral supplementation of plasmalogens twice a day for 28 days improved cognitive tests in certain populations of AD patients; however, this study did not optimize plasmalogen supplementation or elucidate the role of plasmalogens in cognitive function (Fujino et al., 2017).

Here, this study aimed at understanding the role of plasmalogens in response to oxidative stress. More specifically, *Caenorhabditis elegans* (*C. elegans*), was used as the model organism. The *fard-1* mutant was utilized as an AD model, as it lacks the ability to create plasmalogens and was assayed with the oxidative stressors of glucose and galactose. We ultimately examined the differences in the phospholipid population, double bond distribution, and trends in response to the oxidative stressors.

3.0 Literature Review

3.1 Alzheimer Disease

Alzheimer Disease (AD) is a neurodegenerative disorder marked by cognitive and behavioral impairment that significantly interferes with social and occupational functioning (Lakhan, 2022). AD is the most common form of dementia and cause of a decline in cognitive ability (Kumar et al., 2021). The disease is characterized by amyloid plaques and neurofibrillary tangles which are detrimental to the cell (Figure 1). Amyloid plaques are spherical microscopic lesions that have a core of extracellular amyloid beta-peptide surrounded by enlarged axonal endings (Kumar et al., 2021). Plaques come from a fatty transmembrane protein called amyloid precursor protein, APP. In normal neuronal cells, APP is cleaved by either alpha or beta-secretase, and the tiny fragments formed as a result of that are not toxic to neurons. However, in AD, APP is cleaved by beta and then gamma-secretase, resulting in the amyloid plaques, which are toxic to neuronal cells.

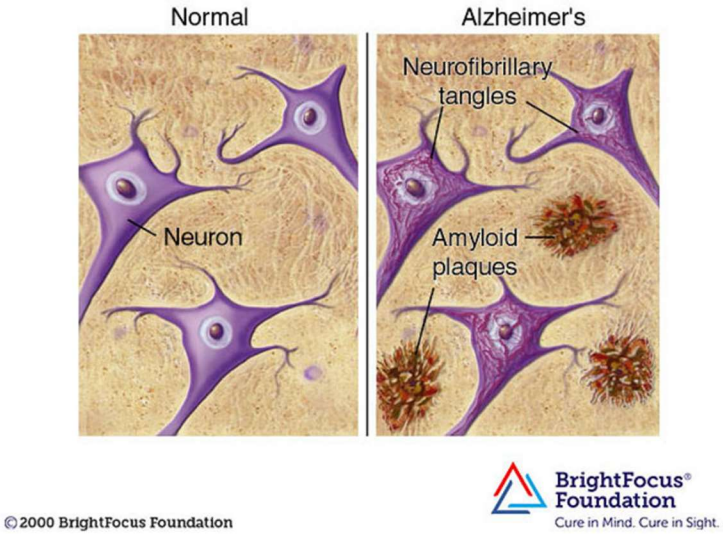


Figure 1: Normal neuronal cells versus Alzheimer cells (Lakhan, 2022)

Neurofibrillary tangles are fibrillary intracytoplasmic structures in neurons formed by a protein termed tau (Kumar et al., 2021). The tau protein stabilizes axonal microtubules, which act as highways for food molecules in the cell. Due to the aggregation from beta-amyloid proteins referred to previously, there is hyperphosphorylation of tau which results in tau aggregates. These aggregates clump together, and form twisted pair helical filaments known as the neurofibrillary tangles which ultimately obstruct microtubule assembly. Therefore, neurons do not get the nutrients they need, resulting in cell death. As mentioned previously, AD does not have a cure. However, multiple studies have demonstrated a decrease in plasmalogens correlated with the severity of AD suggesting that plasmalogens may have therapeutic potential or be utilized as a biomarker.

3.2 Plasmalogens

Plasmalogens are a unique class of membrane glycerophospholipids containing a fatty alcohol with a vinyl-ether bond at the *sn*-1 position and is commonly enriched in PUFAs at the *sn*-2 position of the glycerol backbone (Braverman et al., 2012). These enriched PUFAs are specifically docosahexaenoic, C22:6 or arachidonic acid C20:4 in mammals. In *C.* elegans, an example of a PUFA is C20:5n3. Plasmalogens constitute 20% of the total phospholipid mass in humans with the brain having the highest concentration of plasmalogens constituting 30 mol% of the total the phospholipid population. However, their physiological roles are not clearly understood (Su et al., 2016).

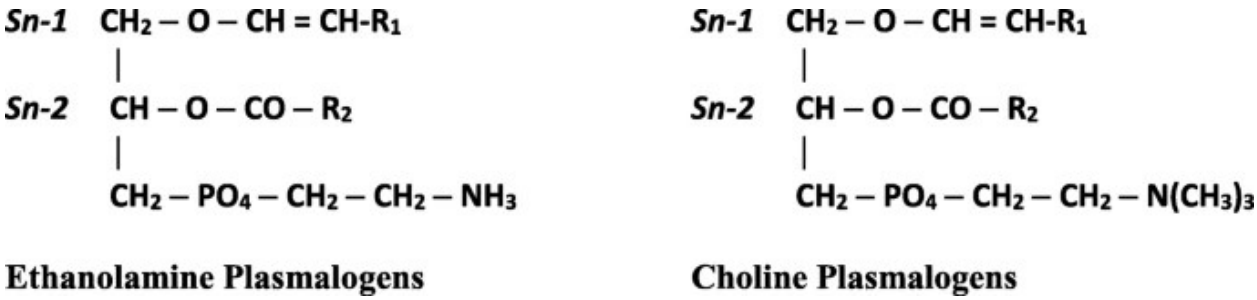


Figure 2: Ethanolamine and Choline Plasmalogen structures (Su et al., 2019)

Plasmalogens have multiple potential functions including contributing to maintaining the membrane physical bilayer properties, facilitating membrane fusion, and signaling transduction processes including cholesterol efflux, preventing oxidative stress, and reducing the inflammatory response (Su et al., 2019). As mentioned previously, perhaps the most relevant function of plasmalogens for this research is their proposed role as sacrificial antioxidants or oxidative scavengers. The vinyl-ether linkage of plasmalogens can serve to trap ROS; it has been demonstrated that interaction between ether lipids and singlet oxygen occurs faster than with other membrane lipids *in vitro* (Broniec et al., 2011). Additionally, when other polyunsaturated phospholipids interact with ROS, reactive peroxides are created which damage the cell and even initiate a cascade of damage, harming the cell further. On the other hand, when plasmalogens get oxidized, they produce less damaging oxidative byproducts including free aldehydes and hydroperoxides (Drechsler et al., 2016).

3.3 AD and Plasmalogens in *C. elegans*

Numerous studies have reported a direct correlation between a decreased level of plasmalogen with the severity of AD. This correlation is of great interest as there is currently no established biomarker for AD allowing for early detection; the decrease in plasmalogens needs to be deeply investigated to reveal its potential use as a diagnostic or prognostic biomarker or novel therapy (Su et al., 2019). In addition to a possible role in disease detection, plasmalogen supplementation therapies may represent potential intervention to prevent disease progression and/or improve symptoms.

It has not been established whether the decrease in plasmalogens is the cause or the consequence of the disease in patients with AD. With that said, there are a few possible mechanisms accounting for the decrease in plasmalogens and how this could contribute to AD described in Figure 2.

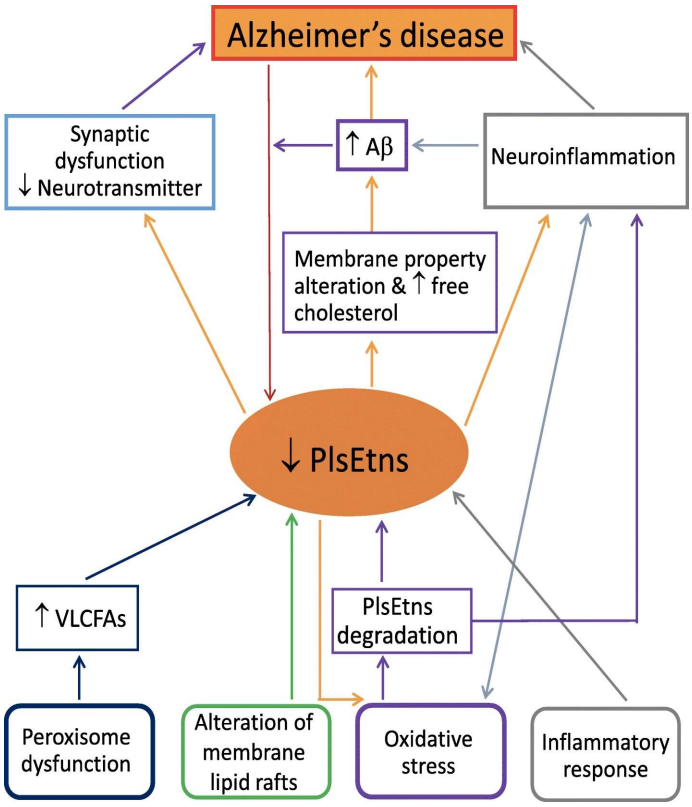


Figure 3: Shi et al. depiction of the proposed relationship between Alzheimer's and plasmalogens.

Oxidative stress is one of the proposed mechanisms attributing for the decrease in plasmalogens and thus AD progression. Aβ are β-amyloid proteins, VLCFAs are very long chain fatty acids, PlsEtns are ethanolamine plasmalogens (Su et al., 2019).

The most relevant reason for loss of plasmalogens could be related to oxidative stress, leading to plasmalogen degradation by reactive oxygen species (ROS). As demonstrated in Figure 4, plasmalogens have a vinyl-ether bond increasing their susceptibility to oxidative damage. The hydrogen atoms adjacent to the vinyl ether bond have relatively low dissociation energies and thus are preferentially oxidized over diacyl phospholipids when exposed to free radicals (Braverman et al., 2012). In a 2021 review, a study demonstrated that in high oxidative conditions, plasmalogen levels were shown to decrease in normal cells, revealing that plasmalogens are functioning as oxidative scavengers (Almsherqi, 2021).

Other proposed mechanisms leading to a decrease in plasmalogens include peroxisome dysfunction, alteration of membrane lipid rafts, or an inflammatory response. Plasmalogens are associated in membrane fusion of synaptic vesicles which facilitates neurotransmitter release. Therefore, plasmalogen deficiency could potentially contribute to synaptic dysfunction and decrease in neurotransmitter seen in AD patients. Further, there is an association between a decrease in plasmalogens and an increase in neuroinflammation which could be related to plasmalogens role as an antioxidant; neuroinflammation is associated with AB accumulation (Su et al., 2019).

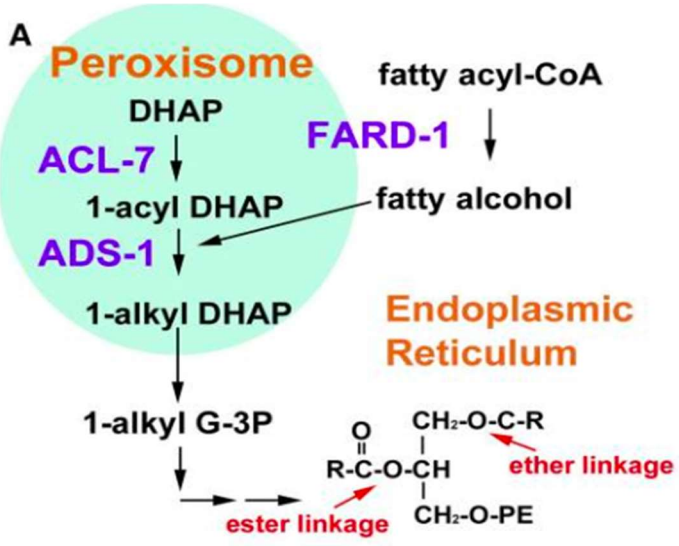


Figure 4: Su et al. demonstrate FAR-1's role in plasmalogen biosynthesis (Shi et al., 2016). *Fard-1* is responsible for converting fatty acyl-CoA to a fatty alcohol. The fatty alcohol enters the peroxisome and contributes to the formation of plasmalogens.

To study AD in the context of decreased plasmalogen levels, plasmalogen deficiency was modeled in *C. elegans* called *fard-1*. *Fard-1* (FAR1 in mammals) is a mutant lacking fatty acyl-CoA reductase which supplies the fatty alcohols that replaces the acyl group; a crucial part of the plasmalogen biosynthesis pathway. Plasmalogen biosynthesis starts in the peroxisome with dihydroxyacetone phosphate (DHAP). DHAP is converted into 1-O-acyl DHAP, and then the acyl chain is replaced by a long chain fatty alcohol from Acyl-CoA synthesis pathway. In this fatty acyl-CoA synthesis pathway, fatty acyl-CoA is converted to fatty alcohol by fatty acyl-CoA reductases (Far1/2). Since Far1 is regulated by negative feedback or cellular plasmalogen levels, it is the rate-limiting step in the plasmalogen biosynthetic pathway (Zhou et al., 2020). As addressed previously, plasmalogens are decreased in AD; *fard-1* models this specific aspect of AD to further understand the role plasmalogens play in AD, and the worm's response to oxidative stress (Shi et al., 2016).

3.4 Membranes are Dynamic Structures

Between the contents of the cell and the external environment, there are 30 angstroms of a hydrophobic barrier called the membrane, where plasmalogens reside. The membrane is mainly composed of molecules known as lipids. These lipids provide the appropriate scaffolding in the membrane for proteins including receptors, transporters, and enzymes. Additionally, the proportions of lipids to other proteins provide the appropriate packing and fluidity that allows the cell to function properly. There are three major classes of membrane lipids molecules: phospholipids, cholesterol, and glycolipids (Fahy et. al, 2011).

The major structural lipids in eukaryotic membranes are phospholipids, also known as, glycerophospholipids. Phospholipids are amphipathic molecules with a polar head group linked to two fatty acid tails. There are different substituents that make up the headgroup such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) etc. PC species are the most abundant comprising 40-50% of total cellular phospholipids including different cell types and organelles. PE species is highly abundant specifically in the mitochondrial inner membranes comprising of 40% of total phospholipids, and only 15-25% of total phospholipids in other organelles (Veen et al., 2017). The fatty acid tail can range from 14 to 24 carbons and vary in the degree of unsaturation from 0 to 6 double bonds in eukaryotes. The different lipid structures including the head group, and the two fatty acid tails create an extremely diverse lipid pool to study. Herein, standard nomenclature (CX:YnZ) will be utilized to describe the fatty acid populations where X indicates the number of carbons in the fatty acid, and the Y shows the number of double bonds at the position Z (Vieira et al., 2022). Further, shorthand notation will be utilized to describe phospholipids. The lipid class abbreviation is proceeded by the fatty acid constituents denoted by (X:Y).

In membranes, phospholipids are arranged in a bilayer; Their head groups are facing the intracellular and extracellular environments, while their hydrophobic fatty acid tails are facing in towards each other. As mentioned previously, PC and PE species are the most common phospholipids in the membrane. PC species have a cylindrical geometry, due to the similar diameter of the head group and their two tails. Thus, they can self-assemble into a planar bilayer contributing to the fluidity of the membrane. PE species contain a small and polar head group, leading to a conical geometry and thus contributing to the curvature of the membrane (van Meer et al., 2008).

Membranes are extremely dynamic structures. They play a variety of roles including vesicle trafficking, phospholipase activity, lysosomal degradation, and beta-oxidation. By responding to these processes, lipids are continually replenished in the membrane; The PE/PC ratio is changing in response to the needs of the cell and the external environment. External environment changes may be an increase in oxidative stressors or temperature fluctuations (Sultana & Olsen 2020).

3.5: Studying Lipids with Mass Spectrometry Lipidomics

As previously discussed, membranes are comprised of diverse lipid species. Lipidomics utilizes high resolution mass spectrometry to characterize this diverse lipid population. There are two main paradigms to analyze lipids with: targeted and non-targeted. Targeted lipid analysis aims at known lipids, and specifically quantifies these specific lipids. Non-targeted lipid analysis is broader in that it focuses on detecting every lipid species simultaneously (Lin et al., 2014). This study primarily utilized a non-targeted approach using liquid and gas chromatography in studying *fard-1* lipidomic's in response to oxidative stress.

Mass spectrometry is coupled with chromatography to further allow separation distinct lipid species. Although liquid and gas chromatography are different strategies, they both reveal valuable insights on the fatty acids in the membrane. Liquid chromatography tandem with mass spectrometry (LC-MS) is a commonly used method to study intact phospholipid composition; it can monitor hundreds of lipids simultaneously (Sultana & Olsen, 2022). Lipids first travel through a reverse phase C18 silica column allowing the lipids to elute based on their increasing hydrophobicity. The wealth of information gained from LC-MS makes it a powerful tool in furthering membrane biology studies.

Along with LC-MS, gas chromatography tandem with mass spectrometry (GC-MS) is a well-known method primarily used to analyze fatty acid tails, identifying regulators of fatty acid metabolism (Sultana & Olsen, 2022). A solid-phase extraction column (SPE) separates the neutral lipids (NLs) and phospholipids (PLs) from the total lipid species before extracting the fatty acids from each class. Before GC-MS can be executed, the fatty acid tails must be converted to more volatile fatty acid methyl esters (FAMEs) (Sultana & Olsen 2019).

Mass spectrometry is then utilized to identify individual lipids with greater molecular and detection specificity. There are two ionization methods utilized in this study: electron and chemical ionization (Lin et al., 2014). In the first, a mass spectrometer ionizes atoms and molecules after gas chromatography with a high-energy electron beam. Then, the ions are deflected through a magnetic field and separated based on the lipids respective mass-to-charge ratios (m/z) (Dancy et al., 2016). In chemical ionization, on the other hand, the analyte collides with the reagent gas with lower energy present in the ion source, generating an intact molecular ion species (Lin et al., 2014).

3.6 *Caenorhabditis elegans*: A Model Organism

Caenorhabditis elegans is a free-living (non-parasitic) nematode worm and is widely used as a model organism for studying several biological processes including apoptosis, cell signaling, cell cycle, cell polarity, gene regulation, metabolism, ageing, and sex determination (Kaletta et al., 2006). The nematode is commonly used for research because they have rapid reproduction with a short generation time; it is relatively small; it is easy to culture. The lifespan of *C. elegans* is relatively short, 2-3 weeks under standard laboratory conditions (Herndon et al., 2018). They are readily raised on the standard diet of *Escherichia coli* or *E. coli*. This standard *C. elegans* diet can be fully stable isotope labeled to monitor lipid dynamics extensively (Sultana & Olsen 2019). The nematode also reproduces rapidly undergoing its full development from egg to an adult as demonstrated in Figure 4 (Kaletta et al., 2006).

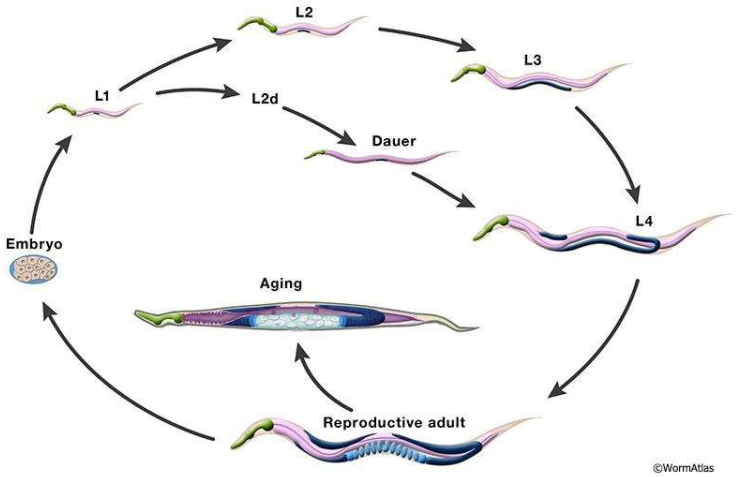


Figure 5: The *C. elegans* life cycle (Herndon, 2022).

The soil nematode emulates certain aspects of human pathology and is often used to identify underlying molecular mechanisms to further ameliorate and/or discover therapeutic strategies. Importantly, the nematode has a similar electron transport system to those of mammals in terms

or its size and genetic contents (Moreno-Arriola et al, 2014). Additionally, the nematode is the first animal for which its genome was completely sequenced (Moreno-Arriola et al, 2014). Since then, WormBase, a database containing *C. elegans* genetic and genomic information curated by a consortium of research, was developed and is now a powerful tool in enabling research on the nematode (Kaletta et al., 2006). Despite this, there are a few differences between the nematode and mammals; *C. elegans* do not have very long-chain fatty acids containing more than 20 carbons while these species are prevalent in mammals (Sultana & Olsen 2019). Also, the nematode lacks cholesterol, limiting research on this aspect of the membrane but allowing for specific interrogation of the role of PUFAs in the membrane.

3.7 Glucose and Galactose Oxidative Stress

High glucose concentrations have been characterized to increase the oxidative load in the cell. Glucose gets oxidized via the tricarboxylic acid cycle (TCA), generating electron donors NADH and $FADH_2$. These electron donors get sent to the respiratory chain (RC) and ultimately overproduces reactive oxygen species (ROS). The ROS overproduction consequently produces an adverse response and damages the mitochondria, thus the cell. The principal ROS include the superoxide anion O_2^-, hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•) (Moreno-Arriola et al, 2014). Reducing sugars, namely glucose and galactose, react non-enzymatically with amino groups in proteins, lipids, and nucleic acids ultimately producing glycation end-products (AGE). AGE are glycated biomolecules as a direct result of reacting with a reducing sugar. The formation of AGE induces ROS as well as depleting nitric oxide leading to an increase in oxidative load in the cell (Singh et al., 2001).

Shi et al., found that plasmalogen deficient *C. elegans* were sensitive to oxidative stress by two chemicals, methyl viologen and tert-butyl hydroperoxide (tBOOH). This oxidative stress can cause lipid peroxidation (LPO) which is the chain of reactions where ROS initiate oxidative damage to lipids ultimately leading to a nonapoptotic form of cell death called ferroptosis. Highly PUFAs are particularly prone to LPO and as previously stated, plasmalogens are thought to protect the membrane against LPO. Ferroptosis occurs from an imbalance between the production and scavenging phospholipid hydroperoxide (PLOOH) and lipid radicals. With this said, plasmalogens have been found to protect from ferroptosis, as previously stated, and act as a ferroptosis sensitizers. Several genes involved in peroxisomal plasmalogen biosynthesis were identified to be the top hits in a genome-wide screen determining ferroptosis sensitivity in cells (Aldrovandi et al., 2021). Interestingly, PE species have been found to be specifically oxidized, despite PC lipids are more abundant in the membrane. This is thought to be related to the fact that PE species are more abundant in the inner leaflet of the membrane (Aldrovandi et al, 2021).

Previous works in the Olsen laboratory aimed at furthering our understanding of the mechanisms required to restore membrane integrity in response to a high glucose diet. It has been established that high-glucose stress perturbs membrane properties by decreasing membrane fluidity. They

found a novel role for monomethyl branched-chain fatty acids (mmBCFAs). The role of mmBCFAS is not in responding to fluidity but the signaling pathway caused by a high glucose environment.

4.0 Materials & Methods

4.1 *C. elegans* Strains and Growth Conditions

N2, or wildtype, and BX275 *fard-1* (wa28; G261D) strains of *C. elegans* were obtained from the Caenorhabditis Genetics Center (CGC). The strains were maintained on 10-centimeter High-Growth, or HG plates seeded with living *E. coli* strain OP50 at 20℃ unless otherwise stated.

To ensure a synchronous population of animals, a process called bleaching was utilized. Every 96 hours, gravid *C. elegans'* adults were subjected to dilute bleach, and the washed eggs were left rotating overnight at 20°C in M9, a salt solution.

Between every experiment, the animals were washed with an M9 solution. Before sample collection, the animals were washed using gravity separation to collect only the adults and exclude the less dense eggs.

4.2 HG, HG Glucose, HG Galactose plates

400 mL HG plates were made by mixing 1.2 grams of NaCl, 8 g of Peptone, and 10 grams of agar. 390 mL of water was added to the mixture. Once autoclaved, 1.6mL of cholesterol and 10 mL of PPB were added. The plates were poured in 10cm petri dishes. For the HG glucose and galactose plates were made the same with two differences. Instead of adding 390 mLs of water, it was 380 mLs of water. After autoclaving and adding the cholesterol and PPB, 10 mL of dissolved 3.24 grams of glucose or galactose was added to the solution, making a total 400mLs solution. The plates were subsequently poured in 10 cm plates. The plates with 45mM of glucose will be referred to as “+gluc” plates, while the plates with 45mM of galactose will be “+gal.”

4.3 Glucose and Galactose Exposure

Synchronized L1’s were placed on regular HG plates for 48 hours. After 48 hours, the animals were collected and washed 3 times before being placed on the previously prepared glucose and galactose plates. These experimental plates were all seeded 4 days prior to the start of the experiment. Approximately 10,000 worms per condition were left to grow for 24 hours until collected. The animals were washed using gravity separation and ultimately collected in Eppendorf tubes and stored in -80°C before processing. In the end, approximately 8,000 animals were collected, and 7,500 processed for analysis.

4.4 Lipid Separation for HPLC-MS/MS Analysis

First, frozen samples were taken out of the freezer to thaw. Once thawed, they were added to 4mL of chloroform/methanol (2:1). 20µL of 0.05 mg/mL internal lipid standard (PC 11:0, TAG 13:0) was added to all samples. TAG is triacyl glycerides which are the major fat storage or neutral lipids in the animals. The samples were left to rotate for 1.5 hours at room temperature. 600µL of 0.9% NaCl was added to clean up the sample; the samples were vortexed and centrifuged at 2000 rpm for 2 minutes. The bottom phases were placed into fresh tubes and dried down with nitrogen steam. 200µL of acetonitrile/2-propanol/water (65:30:5 v/v/v) dilution buffer was added to each sample.

4.5 HPLC-MS/MS Method and Lipid Analysis

10µL of the resuspended lipids in the dilution buffer were injected into the HPLC-MS/MS, set to the negative scanning mode for glycerophospholipid detection. The HPLC system was a Dionex UHPLC UltiMate 3000, equipped with a C_{18} Hypersil Gold 2.1 x 50mm, 1.9um column (25002-052130; Thermo Scientific) and a 2.1 mm ID,5 µm Drop-In guard cartridge (25005–012101; Thermo Scientific). The HPLC was coupled with an Dionex UltiMate 3000 RS quaternary pump, a Dionex UltiMate 3000 RS autosampler, and a Q Exactive Orbitrap mass spectrometer from Thermo Scientific with a heated electrospray ionization (HESI) source.

Once the lipids were processed by the HPLC, they were quantified by Orbitrap mass spectrometry. The first step in post analysis was using Lipid Data Analyzer (LDA) software (Version 2.8.0). LDA utilizes exact mass, retention time, and predicted isotope distribution from full-profile, negative-ion-mode MS1 scans to identify lipids. The RAW files were run against the LDA exact mass list for PC, PE, P-PE, P-PC, O-PE, O-PC, LPC, and LPE (Olsen Lab). A 0.1% cutoff was applied to direct analysis towards the major phospholipid species in the animals (Dreschsler et al., 2016).

4.6 Lipid Separation and FAME Creation

The same method as the HPLC-MS/MS method was utilized for total lipid extraction. 800µL 0.9% of NaCl was added to clean up the sample; the samples were vortexed and centrifuged at 2000rpm for 2 minutes. The bottom phases were placed into fresh tubes and dried down with nitrogen. During these 30 minutes, silica columns were pre-equilibrated with 1mL of chloroform 3 times. Once the lipids were dried down with the nitrogen to prevent lipid oxidation, the samples were resuspended in 1mL of chloroform. Neutral lipids and phospholipids were purified utilizing solid phase exchange chromatography. More specifically, the lipid samples in chloroform were transferred to the column and the flowthrough was discarded. To collect the neutral lipids, 1mL of chloroform was eluted 3 times. For the glycolipids, 1mL of 9:1 acetone/methanol was eluted 5 times. For the phospholipids, 1mL of methanol was eluted 3 times with 1mL. After the elution's, each purified lipid class was dried down separately under

nitrogen. 975µL of methanol and 25µL of sulfuric acid was added to each sample to create fatty acid methyl esters (FAMEs) of each lipid class. The samples were placed at 80°C for 1 hour, and vortexed every 15 minutes. Once the baking step ended, 1.5mL of deionized water and 200µL of hexane was added to each sample to extract the FAMEs. The samples were vortexed and centrifuged for 2 minutes at 2000rpm. The samples were snap frozen in a dry ice bath. Once frozen, the hexane layer was poured into a vial with an insert. 1µL of most samples was run through the GC-MS for data collection. 2µL was run for the neutral lipids as their recovery off the SPE column was lower.

4.7 Lipid analysis from GC-MS

For each sample (containing approximately 7,500 worms), GC-MS analysis was conducted to examine the fatty acid composition of the animals from both neutral lipids and phospholipids. Then, the Chromeleon software integrated the area under the curve of each phospholipid species. Using these areas, relative percentage of each major phospholipid was calculated as the area of the specific lipid divided by the total area multiplied by 100.

For all GC-MS and HPLC-MS analysis, values represent the mean, while the error bars represent the standard error of the mean (SEM) of 6 biological replicates. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests.

4.8 Limitations to Experimental Procedures

There were several limitations to the experimental procedures described. The neutral lipids, analyzed by GC-MS, are the first lipids to be extracted, thus more susceptible to being lost in the silica column. Additionally, the analysis is based on relative percentages, thus if a species decreases, then another must increase given that the total is always 100%. Thus, absolute quantification isn't possible using this method of analysis. As mentioned previously, the fatty acids need to be converted into FAMEs resulting in the loss of head groups of those lipids. Although the LC-MS permits the study of intact phospholipids, it also needs reliable and automated software to store the wealth of data it provides, making the method not as accessible (Sultana & Olsen 2020). Finally, in general, changes in the lipid pools might be diluted by the preexisting large quantities of lipids at the start of the glucose stress (Vieria et al., 2022).

5.0 Results

Decreased plasmalogens in *fard-1*

Previous studies have characterized both the neutral lipid and phospholipid populations in *fard-1* by mass spectrometry (Shi et al., 2016). Thus, the first step of this project was to confirm the differences between WT and *fard-1* (*wa28*) mutant animals. The animals grew on control plates seeded with their laboratory diet of *E. coli* (OP50). The lipids were extracted from the sample, and subsequently processed through both GC-MS and HPLC-MS/MS.

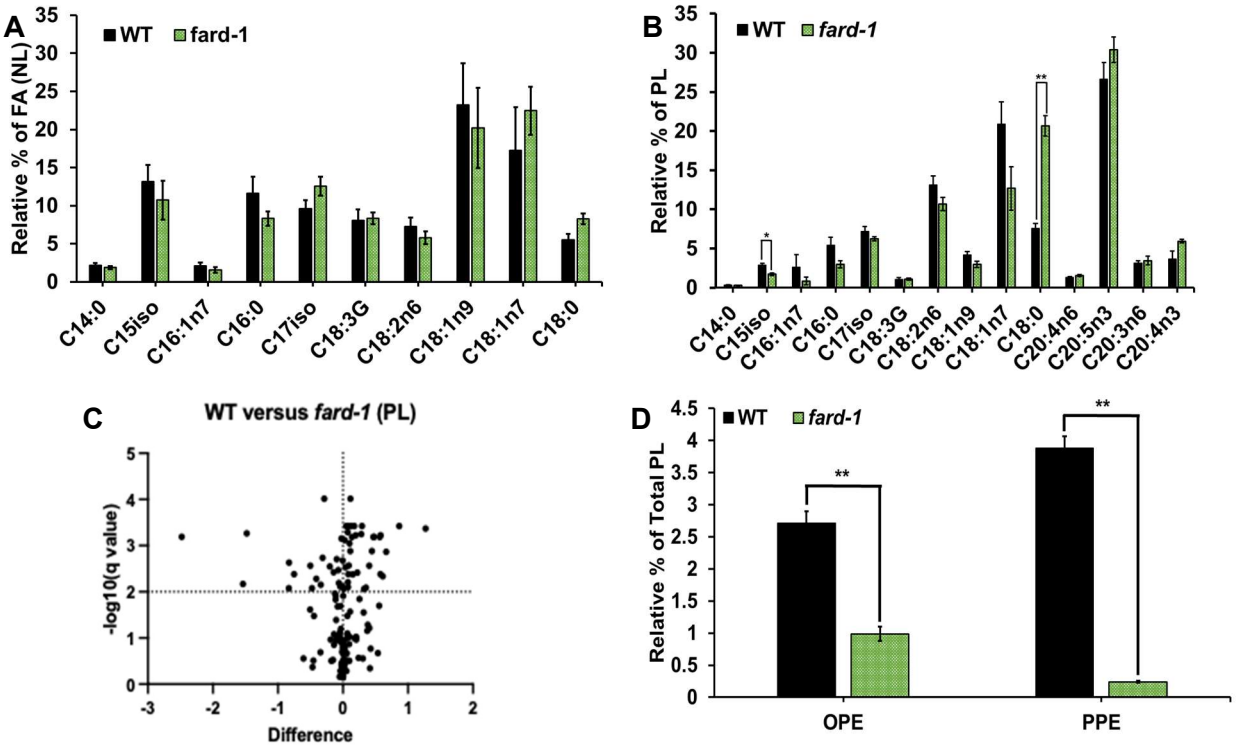


Figure 6. *fard-1* lacks plasmalogens

(A) Total neutral lipid profile between WT and *fard-1* from GC-MS data. (B) Total phospholipid profile between WT and *fard-1* from GC-MS data. (C) Volcano plot generated from HPLC data. Lipids to the right of the vertical line demonstrate an increase, while to the left is a decrease. The further away the black dots are from the horizontal dotted line, the more significant differences they reflect. There is a 36.2% overall change. Of these changes, 65.5% of the lipid species increase while 34.5% decrease. (D) HPLC-MS/MS analysis show that *fard-1* has dramatically reduced plasmalogen levels compared to WT**.** OPE and PPE are plasmanyl ethanolamine and plasmenyl ethanolamine, respectively. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM), and all data represents n=6.

First, the fatty acids from purified neutral lipid and phospholipid populations were analyzed. Figure 6A illustrates no change in the neutral lipid population, while there is a significant decrease in C15iso and increase in C18:0 in the phospholipids, seen in Figure 6B. Previous studies have seen a 3- 4- fold increase change specifically with C18:0 also known as stearic acid (Shi et al., 2016). This increase in C18:0 results from a buildup of that fatty acid population as those fatty acids are no longer incorporated into plasmalogens, thus this analysis suggests that *fard*-1 animals may have a plasmalogen deficiency. To validate this finding, HPLC-MS/MS was utilized to directly measure plasmalogen populations. Figure 6C is the HPLC-MS/MS data demonstrating significant differences in *fard-1*'s plasmalogen population. The specific plasmalogen differences that were identified in the PPE species are 36:3, 36:2, 38:1, 35:1, 38:5, 37:2, 33:0. The differences found in OPE species are 37:4, 34:1, 35:1, 37:2, 36:1, 33:0, 37:1, 36:2. Figure 6D is reflective of the relative percentage of the plasmalogen species compared with the rest of the intact phospholipid pool; it demonstrates that there is significantly less plasmalogen species in the *fard-1* animals compared to N2.

Fard-1 compensating with an increase in PE species

Once the decrease in plasmalogens was established and consistent with literature in the *fard-1* animals, we next wanted to determine if the other phospholipid populations were altered to compensate for the lack of plasmalogens. To do so, the data set was reanalyzed without the plasmalogen population to directly compare the abundance of PC and PE lipids.

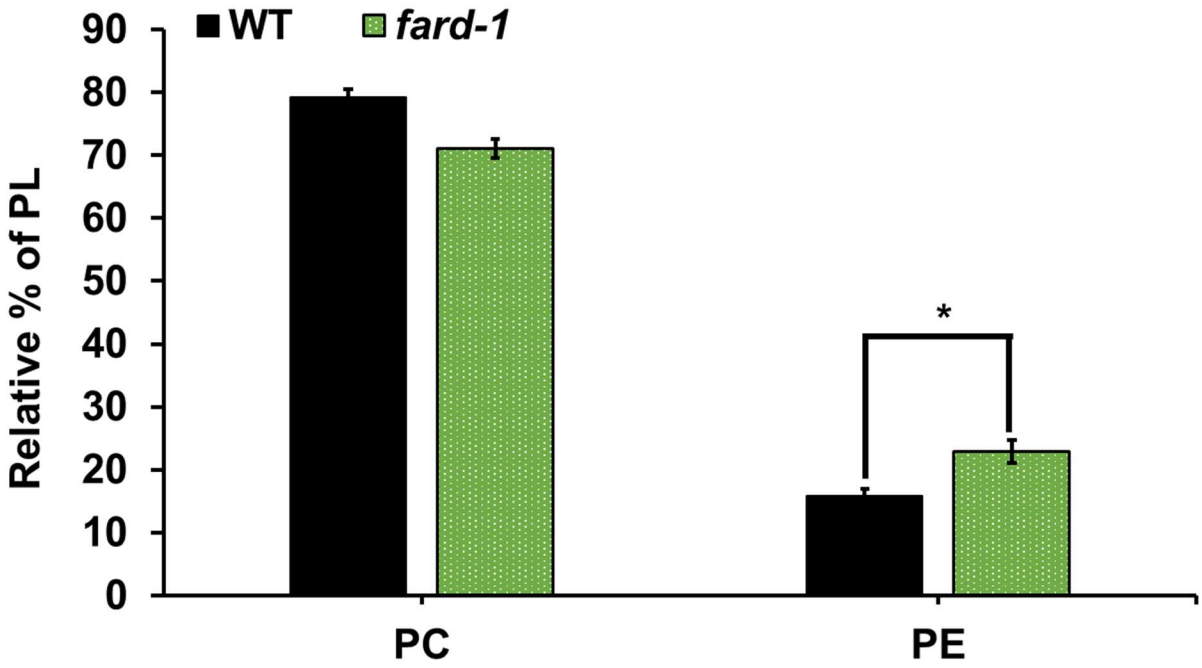


Figure 7. *fard-1* compensates with an increase in PE species

Plasmalogens were removed from the phospholipid's relative percentage calculation. The relative percentage of the phospholipid percentage is quantified in the PC and PE species. These were subjected to statistical analysis. There is a significant increase in PE species in *fard-1* animals compared to WT. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired t-tests. Error bars represent the standard error of the mean (SEM), and all data represents n=6.

In doing so, the *fard-1* worms accumulate a higher amount of PE lipids compared to WT (Figure 7). This most likely indicates that the headgroups that would be used for plasmalogen synthesis are utilized to build ester-linked phosphatidylethanolamine species. This shift would allow the distribution of PC to PE headgroups to remain more constant despite the inability to synthesize plasmalogens.

Fard-1 have decreased PUFAs in PE species

Plasmalogens have been hypothesized to control the overall amount of oxidative stress in a cell by consuming ROS without generating more radicals. In that case, the loss of plasmalogens would make the PUFAs of the membrane more susceptible to damage. It has been shown that the likelihood of LPO increases as the number of double bonds increases, oxidatively damaging the cell (Aldrovandi et al., 2021). To further understand Figure 7 and the impact of the loss of plasmalogens in *fard-1* animals on membrane composition, PC and PE species were binned by the total number of double bonds present to give insight on how the membrane is dealing with oxidative stress.

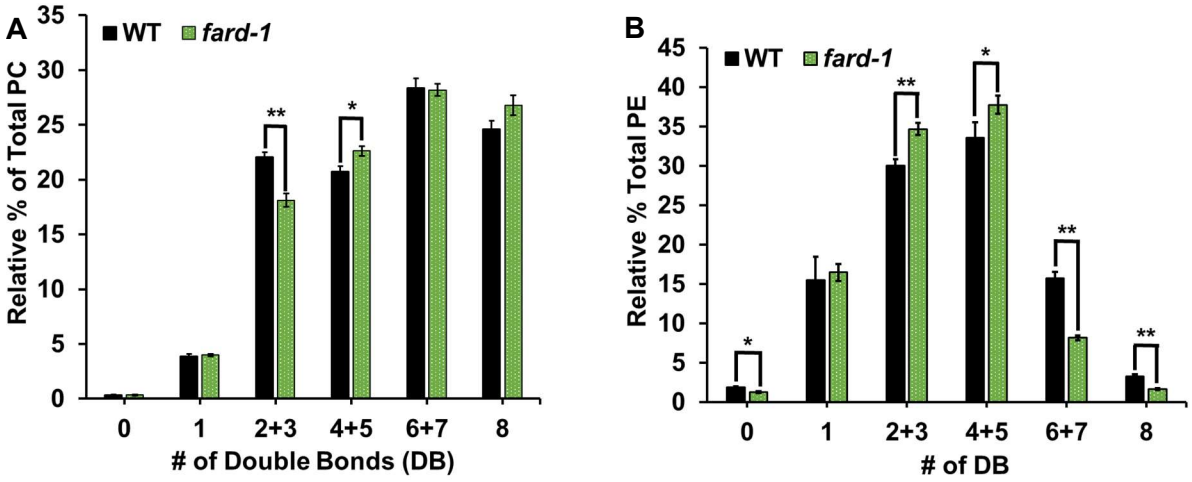


Figure 8. *fard-1* decreases polyunsaturated fats and increases moderately unsaturated fats

(A) Relative percentage versus double bond categories (0, 1, 2+3, etc) in PC species (B) and the PE species from HPLC-MS/MS data. The number of double bonds was summed for both fatty acid chains in the phospholipid. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM), and all data represents n=6.

The results show that *fard-1* animals have a decrease in PE species with 6 or more double bonds indicating a loss in highly PUFA species. Meanwhile, there is an increase in PUFAs with 2-5 double bonds in the PE species. The PC species demonstrate a decrease in lipids with 2-3 double bonds and an increase in lipids with 4-5 double bonds. Overall, there is a loss in highly polyunsaturated species and a compensation with less polyunsaturated lipids (i.e. less than 5 double bonds). This shift suggests a higher amount of damage to the lipids of the membrane particularly the PE population. As mentioned previously, studies have established that there is asymmetry across the lipid bilayer (Fahy et al., 2011); PC species are most abundant in the outer leaflet in many cell types while the PE species are primarily in the inner leaflet of the mitochondria and therefore when intracellular levels of ROS are high, PE species are likely to be impacted from the damage of ROS compared to PC (Aldrovandi et al., 2021). When there is

additional oxidative stress, there is mitochondrial dysfunction defined as diminished mitochondrial biogenesis, altered membrane potential, and the decrease in mitochondrial number and altered activities of oxidative proteins due to the accumulation of ROS in cells (Bhatti et al., 2016). In addition, PE species are commonly found in the inner leaflet of the mitochondrial inner membrane, these lipids would be more susceptible to loss by ROS damage. In addition, due to PUFA's being more susceptible to LPO, the cell may decrease their production and lean more heavily on moderately polyunsaturated fats to mitigate the oxidative damage caused by the environment.

Glucose and galactose impact on *fard-1* animal's

Because the profiles of the *fard*-1 animals suggest a higher degree of oxidative damage without plasmalogens, we next probed whether providing an exogenous stressor would exacerbate that phenotype. Previous works have demonstrated glucose as an oxidative stressor (Vieria et al., 2022), and the next step was quantifying the phospholipid population in response to this stress. PC and PE species were binned by the total number of double bonds present generated from HPLC, orbitrap, and statistical analysis. In addition, there have been limited published works establishing galactose, another sugar, as an oxidative stressor, thus this was executed as well. Figure 9 reflects how *fard-1* is responding to the oxidative stressor's glucose and galactose.

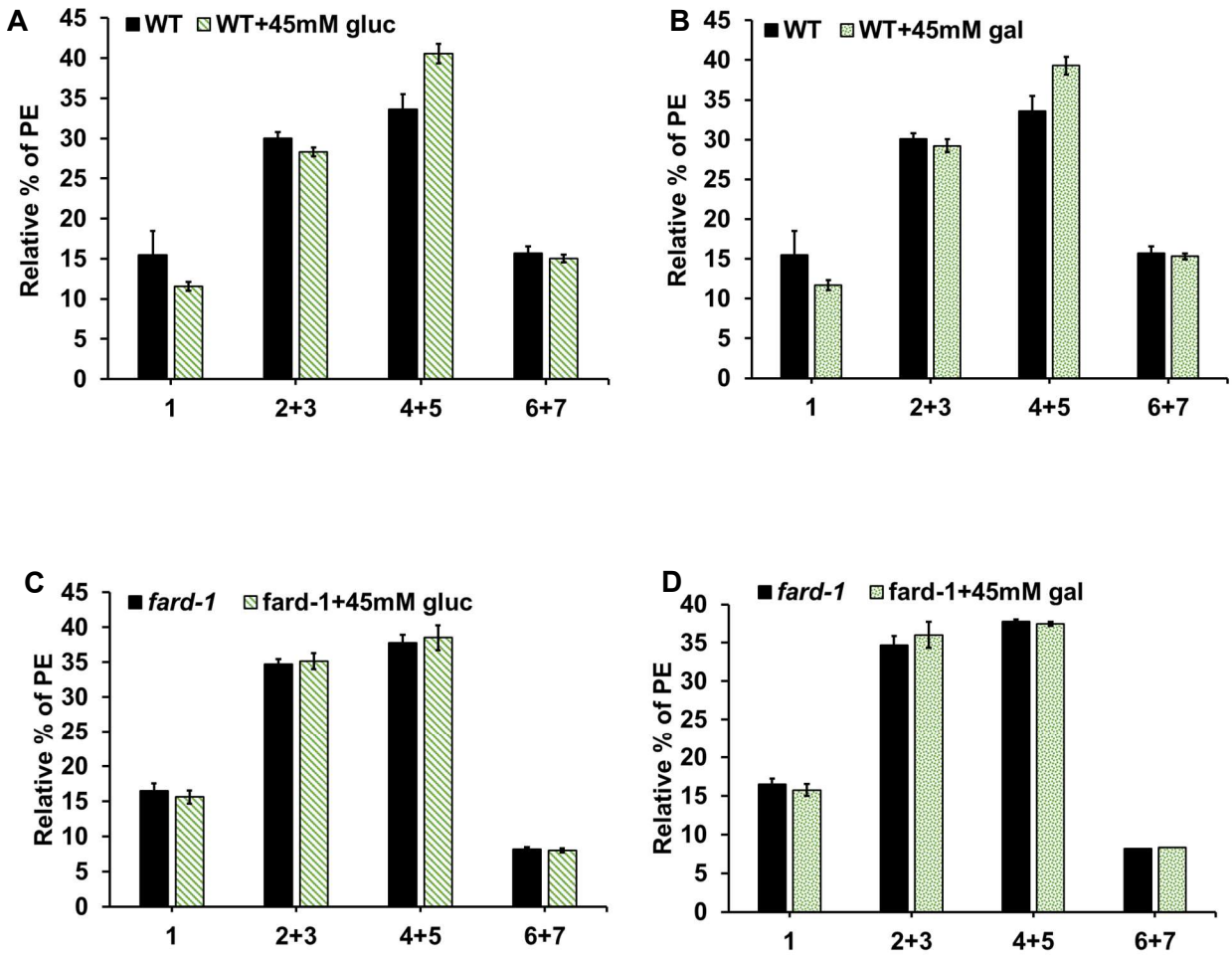


Figure 9. *Fard-1* lacks a decrease in monounsaturated fat and increase in moderately unsaturated fats.

(A)WT versus WT exposed to 45mM glucose for 24 hours show two trends: a decrease in monounsaturated fats and an increase in PE species with 4-5 double bonds. (B) WT versus WT exposed to 45mM galactose for 24 hours show the same trends: decrease in monounsaturated fats

and an increase in PE species with 4-5 double bonds. (C) *fard-1* versus *fard-1* exposed to 45mM glucose for 24 hours; no notable trends to discuss. (D) *fard-1* versus *fard-1* exposed to 45mM galactose for 24 hours; no notable trends to discuss. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM), and all data represents n=6.

Although nothing was found statistically significant, there are two trends to point out. First, there is a decrease in monounsaturated fats in both glucose and galactose in the WT, but the same cannot be said for *fard-1.* Also, there is an increase in lipids with 4-5 double bonds in the WT for both stressors but not for *fard-1.* Ultimately, no changes were found from this analysis in *fard-1* in response to additional oxidative stress. This suggests that *fard-1*'s plasmalogen deficiency does not significantly contribute to the alterations the membrane makes when faced with additional oxidative stressors. This could be attributed to the duration of the oxidative stress: 24 hours. In 24 hours, young adult *C. elegans* can fully replenish their membrane, as their lipid metabolism is significantly faster. However, when these animals grow older, their metabolism slows down, and the effects of the oxidative stressors, glucose and galactose, may be more elucidated.

Glucose and galactose impact on the fatty acid composition of fard-1 a

PUFA are more susceptible to lipid peroxidation, LPO, creating free radicals and thus damaging the cell; plasmalogens do not create the same radicals and alternatively create free aldehydes and hydroperoxides, which cause less damage to the cell. Because *fard-1* PUFA loss in the HPLC-MS/MS data, we examined the PUFA populations directly through GC-MS followed by statistical analysis.

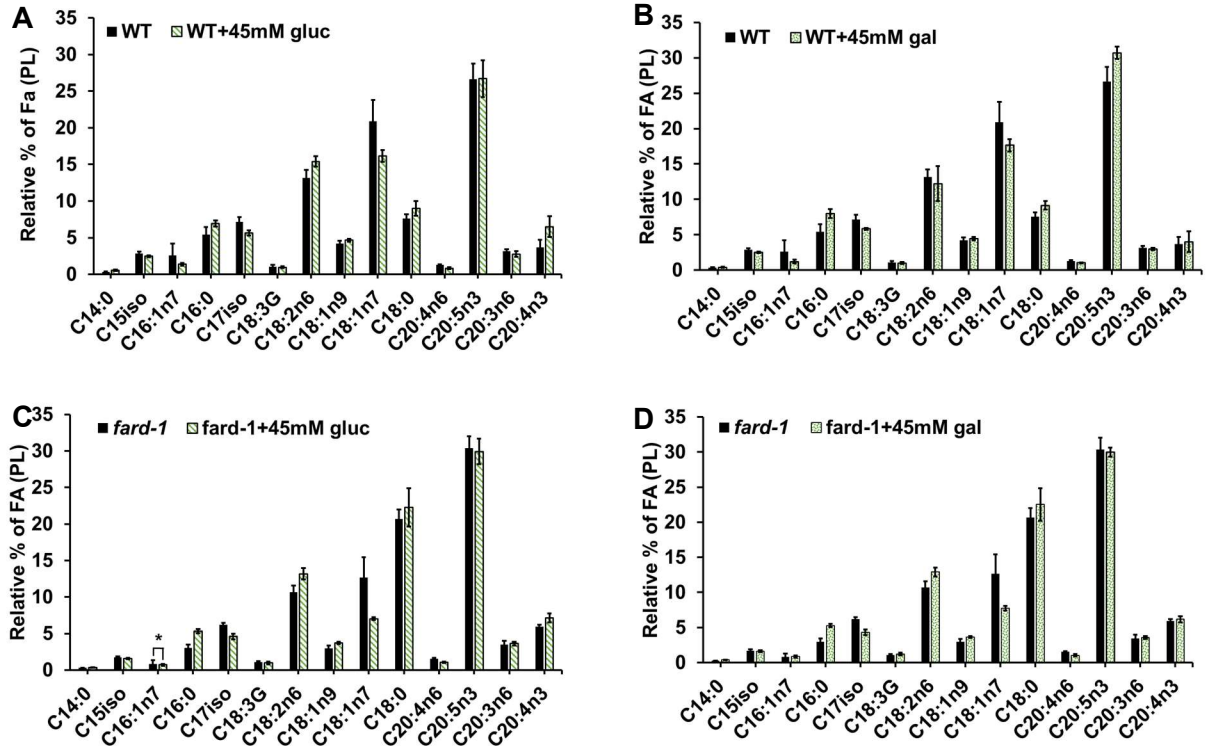


Figure 10. Similar trends comparing glucose and galactose

(A) WT and WT exposed to 45 millimolar (mM) of glucose for 24 hours. (B) WT versus WT with 45mM of galactose stress for 24 hours. (C) *fard-1* compared with *fard-1* with glucose, the same concentration as denoted for WT. (D) *fard-1* compared with *fard-1* with galactose. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM), and all data represents n=6.

Previous studies have characterized an increase in C16:0 in the WT in response to glucose (Vieria et al., 2022). Figure 10 demonstrates this change as well, including galactose stress. Also, we see this increase in the *fard*-1's response to glucose and galactose. Taken together, the lack of plasmalogens in *fard-1* do not seem to significantly be changing the membrane. The young *fard-1* animals have a fast metabolism that might not require plasmalogens to maintain their membrane.

6.0 Discussion

The first step for this project was to establish the differences between N2 or wildtype (WT) and *fard-1* in the context of the laboratory. Figure 6 demonstrates a significant reduction in the plasmalogen species in *fard-1* compared with the WT. The next question to answer was how fard-1 compensates for its lack of plasmalogens.

Figure 7 demonstrates that there is a significant increase in PE species when plasmalogens are taken out of the relative percentage calculation. The working hypothesis relevant to this data is that PC species are enriched in the outer leaflet, while the PE species are preferentially found in the inner leaflet (Aldrovandi et al., 2021). This asymmetry will result in PE species having a higher exposure to ROS and therefore being more susceptible to damage. Because one of the important functions of plasmalogens is as sacrificial antioxidants, the loss of plasmalogens would increase the amount of ROS within the cell. When polyunsaturated fats get oxidized, however, the subsequent fragments are toxic to the cell and oxidatively damage the cell via lipid peroxidation, the degradation of PUFA's in response to oxidative stress. Since plasmalogens are lacking, there is an increase in PE species in order to protect the cell from this oxidative damage resulting from the lack of plasmalogens.

Since double bonds play a crucial role in the oxidative stress response, we decided to look more closely at these bonds while the animal was compensating for its deficiency in plasmalogens demonstrated by Figure 8. Starting with the PC results, there is an increase in lipids with 2-3 double bonds and an increase in lipids with 4-5 double bonds. This could be because the cell is upregulating the PE species and thus lipids are being sent to the inner leaflet as a result of plasmalogen loss. With that said, consistent with the inner leaflet hypothesis, there is a decrease in the more highly polyunsaturated fatty acids and an increase in the more moderately polyunsaturated fats. This would be to decrease oxidative damage to the cell by both the natural oxidative stressors in the environment but also the oxidative damage created by the fragment of the oxidized polyunsaturated fats.

Now that we have established how *fard-1* is dealing with the natural oxidative stressors of a regular environment, Figure 9 demonstrates the results when *fard-1* is faced with additional oxidative stressors glucose and galactose. There are two trends that are most notable: the lack of decrease in monounsaturated fats and increase in unsaturated fats with 4-5 double bonds. This trend suggests that the compensating factors, specifically the upregulation of moderately unsaturated fats versus polyunsaturated fats may be handling the oxidative stress better than the WT does. Since *fard-1* has a decrease in PUFA species, their membrane has a lower peroxidation index compared to WT, thus may be able to deal with peroxidation better than WT.

An alternative hypothesis could be that the oxidative stress glucose and galactose stress was not long enough to see changes in the *fard*-1's membrane phospholipid population. The oxidative

stress lasted for 24 hours. It has been previously stated that the membrane renews itself almost entirely in 24 hours in young adult *C. elegans* (Dancy et al., 2016). Because of this, the young *fard-1* adults could be compensating enough in response to the oxidative stress, while the WT are relying on another mechanism accounting for the changes seen in WT. To address this hypothesis, a lifespan analysis could be done to examine how *fard-1* is dealing with oxidative stressors over time. Also, there should be further analysis done by varying the duration and concentration of the glucose and galactose stress. For example, doing both 15mM and 100mM and increasing and decreasing the amount of time *fard-1* is subjected to oxidative stress.

References

Alcántar-Fernández, J., Navarro, R. E., Salazar-Martínez, A. M., Pérez-Andrade, M. E., & Miranda-Ríos, J. (2018, July 10). *Caenorhabditis elegans respond to high-glucose diets through a network of stress-responsive transcription factors*. PLOS ONE. Retrieved April 28, 2022, from https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0199888#sec020

Aldrovandi, M., Fedorova, M., & Conrad, M. (2021, May 11). *Juggling with lipids, a game of Russian roulette*. Trends in Endocrinology & Metabolism. Retrieved April 25, 2022, from https://www.sciencedirect.com/science/article/pii/S1043276021001004?via%3Dihub

Almsherqi, Z. A. (2021 January 1). *Potential role of plasmalogens in the modulation of Biomembrane Morphology*. Frontiers. Retrieved April 23, 2022, from https://www.frontiersin.org/articles/10.3389/fcell.2021.673917/full#:~:text=More%20importantly%2C%20plasmalogens%20play%20a,and%20lipoproteins%20from%20oxidative%20stress.

Bhatti, J. S., Bhatti, G. K., & Reddy, P. H. (2016, November 9). *Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies*. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. Retrieved April 27, 2022, from https://www.sciencedirect.com/science/article/pii/S0925443916302927

Broniec A; Klosinski R; Pawlak A; Wrona-Krol M; Thompson D; Sarna T; (2011, April 1). *Interactions of plasmalogens and their diacyl analogs with singlet oxygen in selected model systems*. Free radical biology & medicine. Retrieved April 28, 2022, from https://pubmed.ncbi.nlm.nih.gov/21236336/#:~:text=The%20results%20show%20that%20singlet,2%20orders%20of%20magnitude%20greater.

Braverman, N. E., & Moser, A. B. (2012, May 22). *Functions of plasmalogen lipids in health and disease*. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. Retrieved April 21, 2022, from

Dancy, B. C. R., Chen, S.-W., Drechsler, R., Gafken, P. R., & Olsen, C. P. (2015, November 3). *13C- and 15N-labeling strategies combined with mass spectrometry comprehensively quantify phospholipid dynamics in C. elegans*. PLOS ONE. Retrieved April 21, 2022, from

Drechsler, R., Chen, S.-W., Dancy, B. C. R., Mehrabkhani, L., & Olsen, C. P. (2016, November 28). *HPLC-based mass spectrometry characterizes the phospholipid alterations in ether-linked lipid deficiency models following oxidative stress*. PloS one. Retrieved April 21, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5125691/

Fahy, E., Subramaniam, S., Sud, M., & Cotter, D. (2011, June 16). *Molecular biology of the cell - NCBI bookshelf*. Retrieved April 28, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK21054/

Fujino, T., Yamada, T., Asada, T., Tsuboi, Y., Wakana, C., Mawatari, S., & Kono, S. (2017). *Efficacy and Blood Plasmalogen Changes by Oral Administration of Plasmalogen in Patients with Mild Alzheimer's Disease and Mild Cognitive Impairment: A Multicenter, Randomized, Double-blind, Placebo-controlled Trial*. EBioMedicine, 17, 199–205. https://doi.org/10.1016/j.ebiom.2017.02.012

Herndon. (2022). *Introduction to Aging in C. elegans*. Handbook - introduction to aging in C. elegans. Retrieved April 21, 2022, from https://www.wormatlas.org/aging/introduction/mainframe.htm

Kaletta, T., Hengartner, M. *Finding function in novel targets: C. elegans as a model organism*. Nat Rev Drug Discov 5, 387–399 (2006). https://doi.org/10.1038/nrd2031

Kumar, A., Tsao, J. W., Goyal, A., & Sidhu, J. (2021, August 11). *Alzheimer disease - statpearls - NCBI bookshelf*. Retrieved April 21, 2022, from

Li, L., Han, J., Wang, Z., Liu, J., Wei, J., Xiong, S., & Zhao, Z. (2014, June 11). *Mass spectrometry methodology in lipid analysis*. International journal of molecular sciences. Retrieved April 28, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4100164/

Liu, Q., & Zhang, J. (2014, April 15). *Lipid metabolism in alzheimer's disease - neuroscience bulletin*. SpringerLink. Retrieved April 21, 2022, from https://link.springer.com/article/10.1007/s12264-013-1410-3

Moreno-Arriola, E., Cárdenas-Rodríguez, N., Coballase-Urrutia, E., Pedraza-Chaverri, J., Carmona-Aparicio, L., & Ortega-Cuellar, D. (2014). *Caenorhabditis elegans: A useful model for studying metabolic disorders in which oxidative stress is a contributing factor*. Oxidative medicine and cellular longevity. Retrieved April 25, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052186/

Shaheen E Lakhan, M. D. (2022, March 15). *Alzheimer disease*. Practice Essentials, Background, Anatomy. Retrieved April 21, 2022, from https://emedicine.medscape.com/article/1134817-overview

Shi, X., Tarazona, P., Brock, T. J., Browse, J., Feussner, I., & Watts, J. L. (2016, February). *A caenorhabditis elegans model for ether lipid biosynthesis and function*. Journal of lipid research. Retrieved April 21, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4727422/

Singh, R., Barden, A., Mori, T., & Beilin, L. (2001, February). *Advanced glycation end-products: A review - diabetologia*. SpringerLink. Retrieved April 27, 2022, from https://link.springer.com/article/10.1007/s001250051591

Sisk, T. (2021, June 22). *A lasting legacy: Dupont, C8 contamination and the community of Parkersburg left to grapple with the consequences*. EHN. Retrieved April 20, 2022, from https://www.ehn.org/dupont-c8-parkersburg-2644262065/particle-7

Su, X. Q., Wang, J., & Sinclair, A. J. (2019, April 16). *Plasmalogens and alzheimer's disease: A review - lipids in health and disease*. BioMed Central. Retrieved April 21, 2022, from https://lipidworld.biomedcentral.com/articles/10.1186/s12944-019-1044-1

Sultana, N., & Olsen, C. P. (2020). *Using stable isotope tracers to monitor membrane dynamics in C. elegans*. Chemistry and Physics of Lipids, 233, 104990. doi:10.1016/j.chemphyslip.2020.104990

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008, February). *Membrane lipids: Where they are and how they behave*. Nature News. Retrieved April 21, 2022, from https://www.nature.com/articles/nrm2330

Veen, J. N. van der, Kennelly, J. P., Wan, S., Vance, J. E., Vance, D. E., & Jacobs, R. L. (2017, April 11). *The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease*. Biochimica et Biophysica Acta (BBA) - Biomembranes. Retrieved April 24, 2022, from https://www.sciencedirect.com/science/article/pii/S0005273617301220#:~:text=Although%20mitochondrial%20membranes%20consist%20of,(15%E2%80%9325%25%20of%20total

Vieria, A. F. C., Olsen, C. P., Walhout, A. J. M., Diot, C., Tifeki, H., & Xatse, M. A. (2022). *Monomethyl branched-chain fatty acids are critical for CAENORHABITIS elegans survival in elevated glucose conditions*. The Journal of biological chemistry. Retrieved April 21, 2022, from https://pubmed.ncbi.nlm.nih.gov/34826420

Zhou, Y., Yu, N., Zhao, J., Xie, Z., Yang, Z., & Tian, B. (1AD, January 1). *Advances in the biosynthetic pathways and application potential of plasmalogens in medicine*. Frontiers. Retrieved April 23, 2022, from

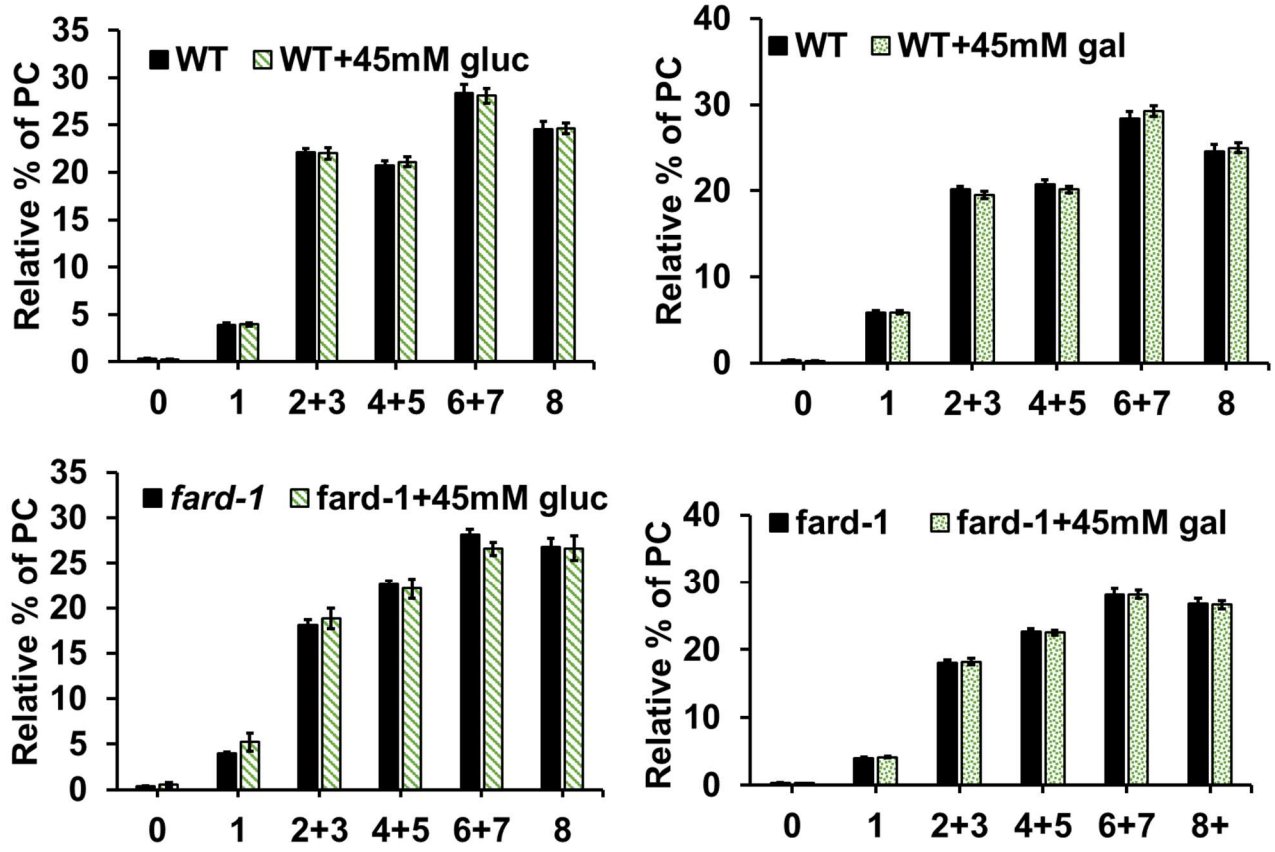
Appendix

Appendix A: Abbreviations used

AD	Alzheimer's Disease
fard-1	Mutant used to model AD in *C. elegans*
C. elegans	*Caenorhabditis elegans*
OP50	*Escherichia coli* strain
LPO	Lipid peroxidation
ROS	Reactive oxygen species
APP	Amyloid precursor protein
Aβ	β-amyloid protein
PlsEtns	Ethanolamine plasmalogens
VLCFAs	Very long chain fatty acids
FA	Fatty acid
TAG	Triacyl glycerides (Neutral lipids)
TCA	Tricarboxylic acid cycle, Krebs cycle, or citric acid cycle
NADH	Nicotinamide adenine dinucleotide
$FADH_2$	Flavin adenine dinucleotide
ER	Endoplasmic reticulum
RC	Respiratory chain
mmBCFAs	Monomethyl branched-chain fatty acids
tBOOH	Tert-Butyl hydroperoxide
PLOOH	Phospholipid hydroperoxide
WT	Wildtype
HG plates	High growth plates
GC-MS	Gas chromatography tandem mass spectrometry

HPLC- MS/MS	High performance liquid chromatography tandem mass spectrometry
LDA	Lipid Data Analyzer
FAMEs	Fatty acid methyl esters
SEM	Standard error of the mean
PE	Phosphatidylethanolamine
PC	Phosphatidylcholine
OPE	Plasmanyl Ethanolamine
PPE	Plasmenyl Ethanolamine
DB	Double bonds
+gal	45 mM galactose
+gluc	45mM glucose
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids

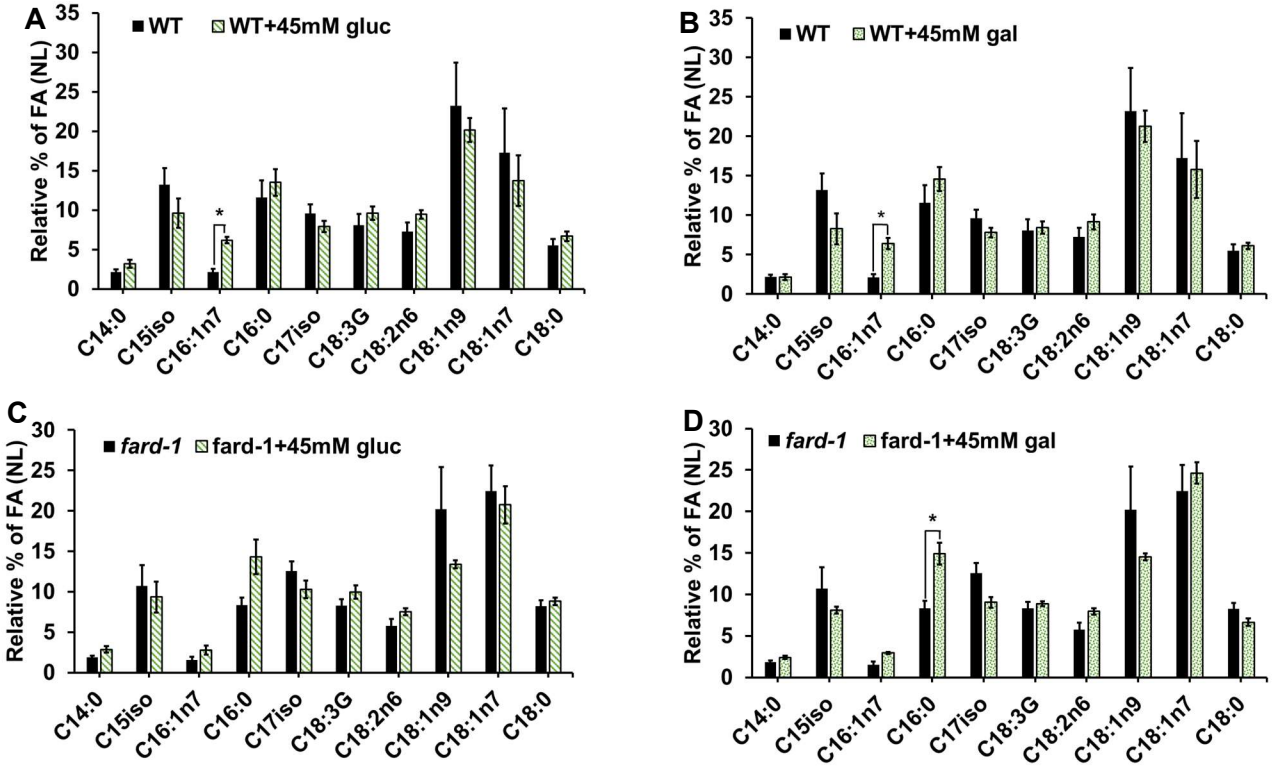
Since the changes we were expecting did not occur in the PE species, we decided to examine the PC species. The PC species are the most abundant phospholipid in the membranes of many different organelles. Once the animals completed 24 hours of oxidative stressors glucose and galactose, they were collected. The lipids were extracted and processed through HPLC-MS/MS. Analysis was done using Orbitrap, mass spectrometry, and statistical analysis.



Appendix B: PC results for glucose and galactose oxidative stress in WT and *fard-1* from HPLC-MS/MS

(A)WT versus WT exposed to 45mM glucose for 24 hours. (B) WT versus WT exposed to 45mM galactose for 24 hours(C) *fard-1* versus *fard-1* exposed to 45mM glucose for 24 hours. (D) *fard-1* versus *fard-1* exposed to 45mM galactose for 24 hours. No notable trends were found in the PC species. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM) and all data represents n=6.

There were no significant changes in the *fard-1* PL population after glucose and galactose stress. We decided to then look at the neutral lipids, being reflective of the storage fat in the membrane.



Appendix C: Neutral lipids after 24 hours of glucose and galactose stress in WT and *fard-1* from GC-MS

(A) WT and WT exposed to 45 millimolar (mM) of glucose for 24 hours. (B) WT versus WT with 45mM of galactose stress for 24 hours. (C) *fard-1* compared with *fard-1* with glucose, the same concentration as denoted for WT. (D) *fard-1* compared with *fard-1* with galactose. Statistical significance, $p < 0.05$ is indicated by * or ** for $p < 0.01$ and was calculated using unpaired T-tests. Error bars represent the standard error of the mean (SEM) and all data represents n=6.

Part 2: Culture of Safety Analysis in New York Hospitals

Author:

Liza Hote, Biochemistry and Professional Writing
Silvana Reid, Biochemistry and Professional Writing

Advisor:

Dr. Brenton Faber, Ph.D., Department of Humanities & Arts
Dr. Yunus Telliel, Ph.D., Department of Humanities & Arts

Culture of Safety at WPI Biomedical Laboratories

At Worcester Polytechnic Institute, we are constantly being educated on the hazards associated with being a lab member and reminded to uphold these safety precautions. This involves lab members adhering to safety protocols including wearing protective personal equipment (PPE) both in academic and research laboratories. Considering the COVID-19 pandemic, we became markedly aware how important PPE, namely wearing face masks, is to protect yourself and those around you. The procedures we had to follow mimicked those often seen in hospitals. Such as doctors must care for their patients, we had to take on a new level of responsibilities for the people around us. Reflecting on our own experiences, we wanted to further explore how to create a culture of safety to instill this in our own lives.

The next piece of this research is focused on analyzing the culture of safety in rural New York hospitals. No previous works have been done to quantify and measure the culture of safety in these areas. In doing this, we hoped to gain further insight into the culture of safety which exists in other medical environments, so we can take away factors they use to apply this in daily life. Our project started with the hypothesis that if hospitals had a poor culture of safety among their staff and employees, then they would also have poor patient outcomes. Through this, we hoped to gain further insight into the patient doctor relationships and how hospitals maintain a culture of safety.

Abstract

Research laboratories are places where hypotheses get assessed and ideas become reality. While labs are the center of innovative science, they contain more hazards and risks than the regular workplace. No matter what research is being conducted, it is vital for any lab member to understand the hazards existing in the environment around them so that they do not put themselves, other lab members, and the research at risk. When completing our separate biochemistry Major Qualifying Projects at Worcester Polytechnic Institute and the University of Massachusetts Medical School were required to obtain training to protect ourselves from chemical, physical, and biological hazards. In relation to this, we explored the outcomes of weak and strong cultures of safety in individual institutions. We hypothesized that if there is a poor culture of safety in any institution, indicated by the number of occupational hazards, there would also be poor work results. Due to access to information, we analyzed New York hospital data in relation to compensation claims and patient outcomes, which were indicative of occupational and patient safety culture, respectively. We then quantitatively correlated the hospital's patient safety grade with occupational hazards in the hospitals. By examining trends and using statistical analysis, we demonstrate that hospitals with better note-taking abilities or ‘measurement robustness,’ tend to have better patient outcomes. The results elucidate ‘you are what you measure,’ and organizations that put the effort into recording their practices, tend to have better work outcomes.

Chapter 1: Introduction

As biochemistry students, we are ingrained with extensive and repetitive training of how important laboratory safety is for both our protection and the overall success of our research. These training sessions cover biological, chemical, and physical hazards which include proper waste disposal and what proper protection equipment is required. However, other institutions may not have the same access or priorities to these kinds of training, and thus have less developed cultures of safety.

The impact that a poor culture of safety can have on employee lives can be seen in the Dupont Teflon crisis. Here, we see an extreme example of how massive corporations allow breaches of safety for their employees in a trade creating monetary gain and sustaining credibility. Every day employees went to their jobs; unaware they were being exposed to toxic chemicals without the proper protective equipment. Hundreds of people's lives were immensely negatively impacted, while Dupont's upper management was acutely aware of the issues but did nothing to prevent it. Similar incidents are found in medical environments, where there are constant reports of a lack of personal protective equipment for medical staff. This was exceptionally seen during the COVID-19 pandemic when many hospital staff went on strike to protest budget reductions that hospital management made which directly affected the well-being of their lives. Other incidents were reported before the pandemic, such as nurses having increased spontaneous abortions through unknowingly being exposed to certain toxic chemicals.

In our research on culture of safety, we were unable to find studies that quantitatively assessed culture of safety in any capacity in hospital settings. As life science students, we found this troubling, as we felt that assigning numerical values to certain culture of safety factors would allow comparison and identification of gaps in safety standards, especially in a clinical environment. Through our examination of New York Hospitals, we quantitatively assessed whether a poor culture of safety is linked to poor patient outcomes. We hypothesized that increased occupational hazards would lead to a worse patient outcome.

To quantify the culture of safety, we created a data set in two-steps. The number of occupational hazards was determined from the NYS Assembled Workers compensation claims dataset. Hospitals don’t often publicly publish any information informing occupational hazards at their institutions. Therefore, we summed the number of occupational hazards by zip code that was self-reported. In tandem with this, we assessed patient outcomes, used the Medicare website, and independently developed a criterion to rank the New York hospitals. The grading criteria were designed to encompass multiple patient outcomes to common procedures to be able to compare hospitals with ease. Using these two datasets in conjunction with other standardization metrics, we proceeded to analyze the numerical values assigned. We determined if there was a correlation between the quality of patient care and the culture of safety for healthcare workers. Based on the results, we identified indicators and causation factors of a poor culture of safety.

Chapter 2: Literature Review

2.1 What is an Occupational Hazard?

Occupational Safety and Health Administration (OSHA) defines an occupational hazard as a risk associated with working in a specific occupation (Department of Labor, 2022). These risks are classified as either long-term, where they directly cause stressors to the body, or short-term, where they cause emotional stress. Both short-term and long-term occupational hazards can directly affect the workplace environment through a decrease in physical and emotional safety. Hazards can be separated into four separate categories: physical, chemical, biological, and ergonomic risk factors. Each of these categories causes varying degrees of long- and short-term impacts, with a large influence stemming from human factors. (Department of Labor, 2022). Human factors are defined by the World Health Organization as "individual characteristics which influence behavior at work in a way which can affect health and safety" such as daily tasks, working environment, policies, and attitudes. Workplace environments that do not properly monitor occupational hazards and allow for an unsafe work environment have proven to have significant negative impacts on the culture of safety and cause detriments to the lives of workers both in and out of the workplace (Department of Labor, 2022). The focus of our project was to assess these culture of safety violations and determine how occupational hazards were interconnected.

2.1.1 DuPont: Making a Toxic Product

Investigating case-studies regarding violations of safety culture helps build an understanding of how and why they occur. Violations of culture of safety due to occupational hazards can notably be seen in the DuPont lawsuit. In 2017, DuPont agreed to pay approximately 617$ million to settle thousands of lawsuits brought against them in relation to their product, Teflon, which was responsible for the widespread intoxication of C8 (Rich 2016). C8 is the common term for Perfluorooctanoic which is per fluorinated carboxylic acid produced and used worldwide as an industrial surfactant in chemical processes and as a material feedstock. Widespread intoxication is a concern because in 2012, after seven years of study, a panel of public health experts released a report documenting a probable link between C8 and six conditions: testicular cancer, kidney cancer, thyroid disease, ulcerative colitis, pregnancy-induced hypertension, and high cholesterol (Sisk 2021). According to a 2007 study, C8 is in the blood of 99.7% Americans, earning the name the 'forever chemical' since it never degrades. Further studies have identified that a majority of the C8 found in the systems of Americans is from Teflon products, such as their non-stick pans (Sisk, 2021).

On record, previous female Dupont employees reported being moved to different departments unexpectedly when they became pregnant. From this group, a percentage of the children born to these employees had physical deformities and demonstrated elevated C8 in their systems. In

addition, many of the workers who were exposed to C8 for extended periods of time developed rare forms of cancer directly linked to C8 intoxication. It was discovered that DuPont had been aware since at least the 1960s that C8 was toxic in animals and since the 1970s that there were high concentrations of it in the blood of its factory workers. DuPont scientists were aware in the early 1990s of links to cancerous tumors from C8 exposure, however, company executives failed to inform the Environmental Protection Agency [EPA] or the public (Sisk, 2021) (Rich, 2016).

Due to failed management and lack of transparency, Dupont created an unsafe workplace environment which affected not only their employees' lives, but also their families and the surrounding town area. Lack of proper chemical management led to the development of increased cancer risk as well as birth defects; casualties could have been avoided if management had investigated and created proper safety protocols to decrease exposure to their employees. Dupont exemplifies what a poor culture of safety can cause: an increase in both long- and short-term occupational hazards. This, in turn, has resounding impacts on their employees' lives.

2.1.2 Hazards in the Hospital Environment: Hospital Personnel at Risk

Our study looked directly at the culture of safety in a hospital environment. To do this, we must understand the work environment that exists for clinicians and other hospital personnel. Hospital personnel and staff are exposed daily to various types of occupational hazards such as physical and chemical dangers, radiation, infectious risks, and psychosocial problems prevalent in hospitals (Jachuck et al, 1989) (CEB, 1985). Exposure to toxic chemicals encompasses a wide distribution of hazards since they can range from minor skin irritation to possible carcinogenic (CEB, 1985).

As mentioned previously, nurses face a wide range of workplace hazards including dangerous chemicals and drugs. They also face sharp injuries, back injuries, violence, and stress. Despite consistently being exposed to hazards, nurses and related medical personnel consistently face the lack of personal protection equipment (PPE). During the COVID-19 pandemic, nurses reported a deficiency in face shields and masks. Lack of available PPE puts nurses in direct danger, both from viral infections such as COVID-19 and other hazards found in the workplace (Cohen et al, 2020). Failure to provide this PPE not only puts nurses, but the entirety of the healthcare system at risk as it decreases the effectiveness of caregivers.

Both viral and chemical exposures have been demonstrated to have an impact on the personal lives of nurses. Usage of chemicals such as anesthetic gas is an extremely common procedure, however, without proper PPE can cause harm to the nurses administering it. Female nurses have participated in studies to determine the effects of an antineoplastic drug exposure on birthing rates (Lawson 2013). Out of the 6707 live births included in the study, 775 reported spontaneous abortions 20 weeks before the due date. When factors such as age, parity, shift work, and hours

worked were included, it was determined that excessive exposure with antineoplastic drug was associated with a 2-fold increased risk of spontaneous abortion, particularly with early spontaneous abortion before the 12th week, and 3.5-fold increased risk among nulliparous women (Triolo 1989). In addition, nurses who were exposed to sterilizing agents, such as steam autoclaves, demonstrated a 2-fold increase in late spontaneous abortion (12-20 weeks). Irregular births in nurses due to exposure causes concerns for the occupational safety of them, but also for the safety of the patients they attend to. Decreased occupational safety may be correlated to worse patient care outcomes which may be reflective of the deficient culture of safety within the facility (Fleisher 2022).

2.1.3 Preventive Measures

Most hospitals have an employee health service available to their employees to prevent hazards such as chemical exposure. However, many lack professional training to handle job-related risks and assessing appropriate preventive measures (CEB 1985). To combat this, some states have also passed “right-to-know" laws which require worker education about hazards in their work environment to increase knowledge and decrease accidents. The COVID-19 pandemic actively highlighted declining factors of US health care safety and the lack of resilience in safety culture infrastructure. Consistently, health care workers lacked sufficient materials to safely work in medical settings. Occurrences such as this call into question the maintenance of worker safety and the overall cultural safety of medical environments today (ECRI 2019).

2.2 Culture of Safety in Health Care Systems

A culture of safety is defined as the attitudes and behaviors of the group and individuals toward patient safety within a healthcare facility. A culture of safety can most readily be observed through policy and leadership (ECRI, 2019, 10). Most of the time, a culture of safety is a reference to the relationship between the patient and the medical community made up of doctors, nurses, and other staff. When a patient is taken into care, an entire healthcare team is responsible for patient safety and must work together to ensure patients' well-being during treatment. A key component of ensuring patient safety is to document patient safety outcomes, including adverse events (Han Y et al., 2020). This touches upon another aspect of culture of safety: the ability of an organization to measure, recognize, and correct misidentifications, misspecifications, and misunderstandings that pose threats to safety (Vogus et al 2010). When conducting our research on the notion of ‘culture of safety', we focused on the quantitative portion of the definition: the ability of an organization to measure its patients' outcomes.

2.2.2 Patient Impact

It has previously been shown that an established culture of safety leads to a reduction of adverse events as it pertains to patient safety (Han Y et al., 2020). This has been measured in a variety of dimensions including reduced infection rates, fewer readmissions, better surgical outcomes, reduced adverse events, and decreased mortality (ECRI, 2019, 10). In a 2020 study, researchers Han Yonghee, Kim Ji-Su, and Seo YeJi found and published in the Western Journal of Nursing Research that higher mean scores for "communication openness" in patient safety culture were significantly correlated with lower rates for pressure ulcers and falls. Additionally, higher mean scores for "working in teams with other health professionals" in patient safety competency were significantly correlated with reductions in ventilator-associated pneumonia (Han et al., 2020). While we assume there may be multiple factors involved, we have come to assume that fewer poor patient outcomes demonstrate a greater culture of safety. The quality health care would not be able to be delivered if there is a poor culture of safety, because the employees themselves would not be safe. Several strategies (Weaver et al, 2013) have been used to try to create an improved culture of safety, such as bundling multiple interventions or tools that employees can use as a resource to improve the safety culture. Accreditation bodies identify leadership standards for safety culture measurement and improvement (Guldenmund, 2000).

2.2.3 Culture of Safety Process Improvement

Cultures of safety are directly impacted by strong leadership committed to ensuring safety practices within the organization. This can look a variety of ways: leaders should encourage employees to "learn about errors and near misses, investigate errors and near misses, investigate errors to understand their causes, develop strategies to prevent error recurrence, and share the lessons learned with staff so they recognize the value of reporting their concerns"(ECRI, 2019, 10). One specific aspect of these action recommendations is "reporting their concerns." Creating an environment where employees can report allows mistakes to be caught earlier and thus improved at the facility level.

2.2.4 Barriers to Process Improvement

To improve incident reporting, especially among doctors, clarification is needed of which incidents should be reported to increase simplification and feedback given to reports. An example of this can be seen in a qualitative study of OHS risks and preventative measures which was conducted with a sample of Australian small business to better understand the occupational health and safety of the construction industry. They selected both immediate consequences (such as fall risks) and long-term consequences/health effects (such as skin disease) as analytical factors. Through this, they found that most employees see occupational injury as an unavoidable aspect of their careers (Lingard 2022). In work settings, workers often feel barriers extend to the individual, whereas a lot of the failures are the result of poor management and access to

resources. Additionally, the dualism of physical barriers occupational hazards, but also social barriers, such as group mindset and ostracization. Such barriers can be products of strategic organizational rhetoric and cultural discourse such as coalition formation (Lingard, 2022) (Zoller, 2009) (da Silva, 2019).

Chapter 3: Research Design

Our research was aimed at determining if there was a positive correlation between a culture of safety in the workplace and patient outcomes. This hypothesis can be examined two-fold: occupational hazards and patient outcomes. Patient outcomes were determined using the Medicare website and an independently developed quantitative measurement called "safety grade." This encompasses the ratings each hospital received in common patient procedures. The common patient procedures included the rate of complications of knee/hip replacement patients, serious complications, and death among patients with serious treatable complications after surgery. This data set ultimately served to measure the patient outcomes in selected hospitals in New York. The second part of the research was to examine the culture of safety through the lens of occupational hazards. The number of occupational hazards was calculated from the New York compensation claims to determine how many injuries occurred in these hospitals. Once this was done, the hospital and occupational hazard data sets were combined via zip codes to determine which hospitals had the most or least occupational hazard claims. At the end, we combined both research paths to assess overall relationships between a culture of safety and patient outcomes.

3.1 Analysis of Patient Outcomes

3.1.1 Rating Hospitals: Safety Grade and Data Robustness

We used the Medicare website to collect data about the patient outcomes in New York hospitals (Medicare.gov, 2022). As seen in Figure 1, the Medicare website offers several resources with the goal of informing patients and health care providers about different health and drug plans. We narrowed down the number of hospitals by only looking at emergency services (hospitals that provide emergency services like acute medical or trauma care) and hospital type (acute care) (ECRI, 2019, 10). Once we found this list of hospitals, we devised a rating criterion based on the national rating system the website provided (Table 1) This was done by clicking the hospitals, then clicking the "complications and deaths," tab and rating them based on pre-decided ratings that are based on the national averages seen in Table 1. The specific definitions of the items in the rating column can be found in Appendix B.

Table 1: The rating system utilized in grading the hospitals informing the safety grade metric.

Rating	Below average	Average	Above average	Number of cases too small
Rate of complications for hip/knee replacement patients	-5	0	5	0
Serious complications	-5	0	5	0
Death among patients with serious treatable complications after surgery	-5	0	5	0
CLABSI	-5	0	5	0
CAUTI	-5	0	5	0
SSI Colon	-5	0	5	0
MRSA	-5	0	5	0
C. diff	-5	0	5	0

Once we developed the safety grade criterion, we developed our process of grading the NY hospitals. Figure 1 demonstrates the interface once the hospitals were filtered by both "Emergency Services," and "Acute Care."

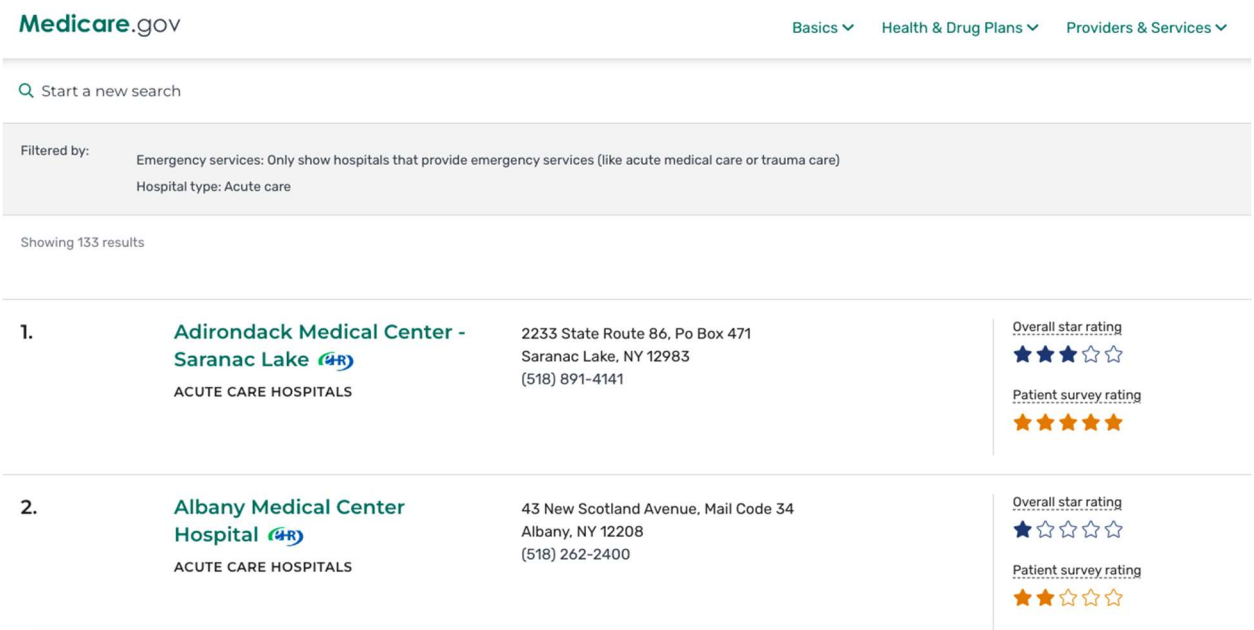


Figure 1: Excerpt from the Medicare website already filtered by hospitals that provide emergency services and acute care.

After filtering the Medicare website, we found 135 hospitals to grade. The hospitals were rated as being above average, or below average. There were also fields: “no reported data,” or “not enough data available”; Examples of these fields are shown in Figure 2. When the field “number of cases too small,” appeared we first rated the hospital negatively. Upon further investigation, this grade was excluded from analysis because we did not want to bias and exclude smaller hospitals that may have less resources to record but could still have good patient outcomes.

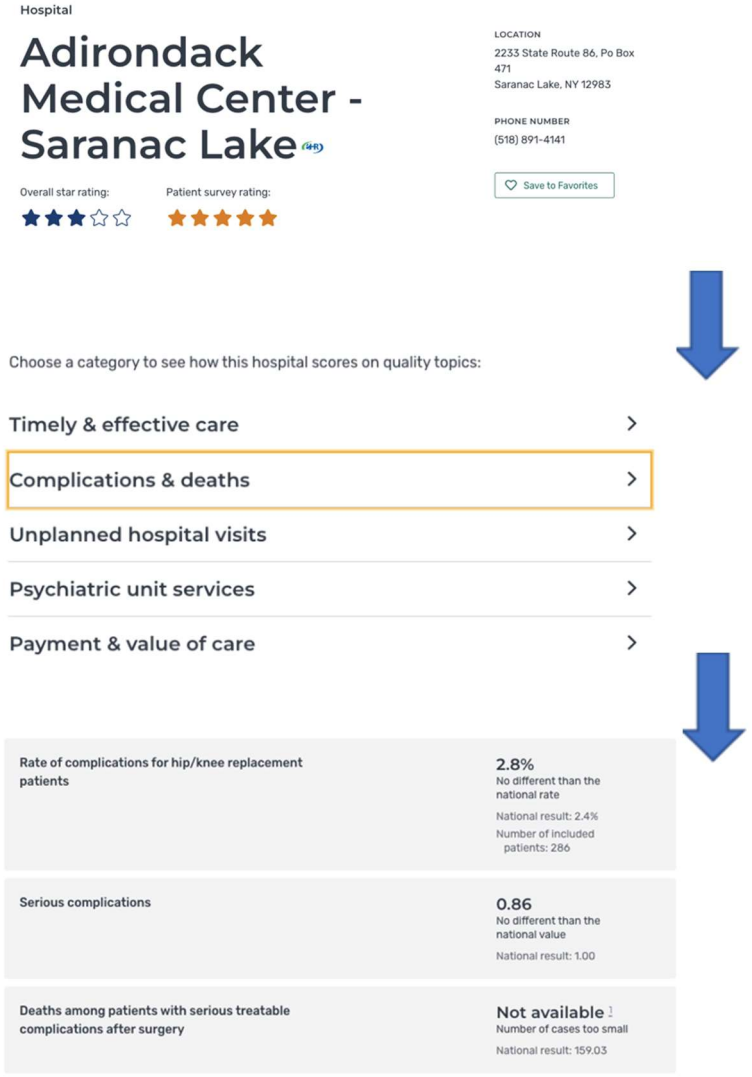


Figure 2: Website interface process to access the common patient rating

The next step was to create a comprehensive Microsoft Excel sheet with the hospitals, zip codes, and grades. The Microsoft Excel sheets were color-coded to stay organized and have a firm understanding of which hospitals were graded higher versus lower to identify outliers or any missing fields. To analyze the quality of the hospitals recorded data, we developed a metric

termed data robustness. This was a qualitative measurement of the hospital's capacity to report their data. It was identified by the number of Medicare categories for which the hospital reports complete data. As mentioned, this field became "data robustness" and was scored 0-6. Hospitals with fully reported data (positive or negative) were scored 4-6 while hospitals with missing and incomplete data were scored lower 0-2. This enabled us to compare quality scores against the robustness of each hospital's reporting system. Although studies have been done to examine patient safety practices, none have attempted to quantitively assess patient outcomes and occupational hazards to inform culture of safety. As a result, we independently developed the data sets based off what was directly available for the public online.

Name of Hospital	Zip Code	Knee/Hip	Serious	Death	CLABSI	CAUTI	SSI colon	MRSA	C Diff	Safety Grade
United Health Services Hospitals, Inc	13903	0	0	0	0	0	0	-2	0	-2
St Mary's Healthcare	12010	0	0	0	0	0	0	-2	0	-2
Newark-Wayne Community Hospital	14513	0	0	0	0	0	-2	-2	0	-4
Upmc Chautauqua at Wca	14701	0	0	-2	0	0	0	0	0	0
Claxton-Hepburn Medical Center	13669	0	0	-2	0	0	-2	-2	0	0
Good Samaritan Hospital of Suffern	10901	0	0	-2	0	0	-2	-2	0	0
Olean General Hospital	14760	0	0	-2	0	0	0	0	0	-2
Oswego Hospital	13126	0	0	0	-2	0	-2	-2	0	-6
Richmond University Medical Center	10310	0	0	0	0	0	0	0	0	0
F F Thompson Hospital	14424	0	0	0	0	0	0	0	0	0
Hudson Valley Hospital Center	10567	0	0	0	0	0	0	0	0	0
St Josephs Medical Center	10701	0	0	0	0	0	-2	-2	0	-4
Kaleida Health	14210	0	0	0	0	5	0	0	5	10

Figure 3: An excerpt from Microsoft Excel recording the New York hospitals, their respective zip codes, and grades from the common procedures.

3.2 Determining Culture of Safety Through Occupational Hazards

We used the NYS Assembled Workers Compensation claims data set to determine the number of occupational hazards in NY hospitals (York, 2021). The NYS assembled workers' compensation claims developed by the Workers' Compensation board (WCB) provides data on worker's compensation, disability, volunteer firefighters', volunteer ambulance workers', and volunteer civil defense worker's benefits. The WCB uses this data to ensure employer compliance and regulates different stakeholders involved including self-insured employers, medical providers, and third-party administrators. The WCB determines claims of workplace injury by injuries that cause a worker to lose more than one week of work, or permanent disability. WCB also includes injuries disputed by the employer, or when they receive a C-3 Form (claim from the injured worker). Although this data set included many fields not related to healthcare providers, we filtered it to only investigate claims recorded under the healthcare field. The process of connecting hospitals to occupational injury count can be seen in Figure 4. By the end of this process, there were 25 hospitals studied.

	Count Occupational Injury	Occupational Injury Zipcode	Hospital
	27	12983	Adirondack Medical Center- Saranac Lake
	1	12977	Adirondack Medical Center- Saranac Lake
	16	12913	Adirondack Medical Center- Saranac Lake
	7	12945	Adirondack Medical Center- Saranac Lake
	27	12946	Adirondack Medical Center- Saranac Lake
	3	12939	Adirondack Medical Center- Saranac Lake
total	81		

Figure 4: Excerpt from the process of counting the occupational zip code count in a 10-mile radius of Adirondack Medical Center-Saranac Lake

3.3 Correlation Factors Between Occupational and Patient Safety

3.3.1 Zip codes

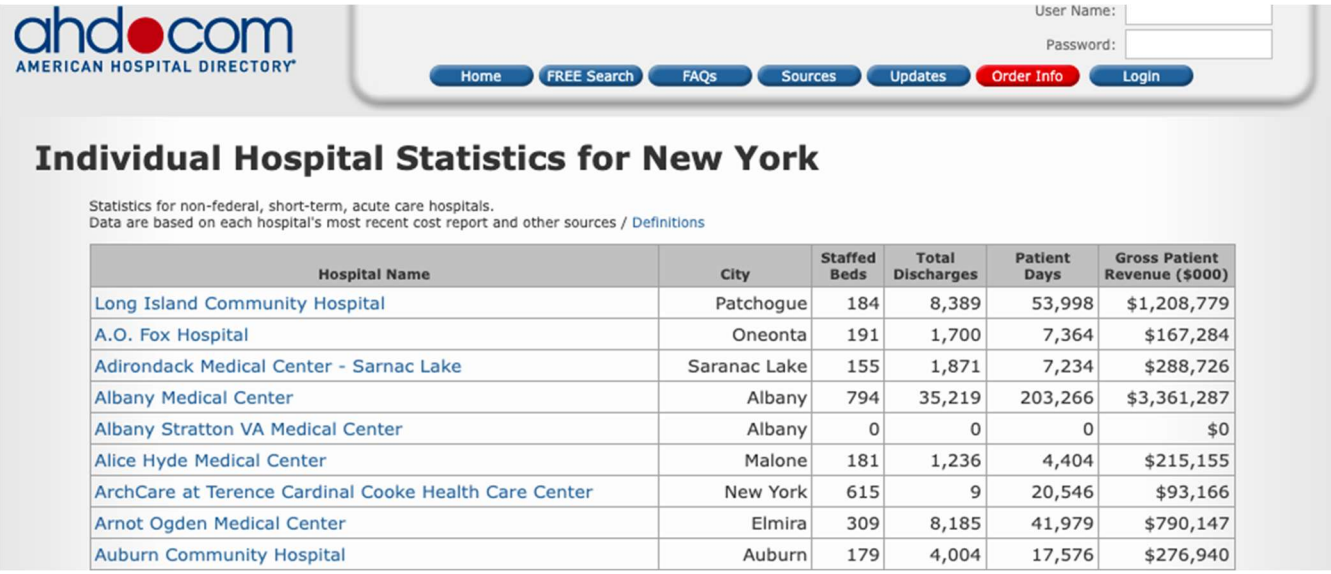
To combine the hospital and the occupational injury data sets, we found the zip codes within a ten-mile radius for the hospitals and matched them with the occupational hazards that occurred at those specific zip codes. We used a zip code query to find these zip codes (Qi, 2019). We counted the number of occupational claims in those zip codes within a 10-mile radius of the hospitals. It should be noted that during this portion of our research, we had excluded hospitals in major cities, New York City, Buffalo, Rochester, and Albany due inability to accurately determine which occupational injury corresponded to which hospital due to zip code overlap.

3.3.2 Population

Hospital size depends on the number of beds but is also impacted by the geographical location. To address this issue and enable accurate hospital comparison, we standardized their size based on the population where the hospitals are respectively located. This was done from a simple Google search to determine population size in each zip code.

3.3.3 Number of beds

The American Hospital Directory was the source utilized to identify the number of beds in the hospitals. The American Hospital Directory provides “data, statistics, and analytics about more than 7,000 hospitals nationwide” (American Hospital Directory, 2022). The number of beds, as seen in the “staffed beds” field in Figure 5, was employed to standardize injury counts, given that it allowed us to determine the size of hospitals.



ahd.com
AMERICAN HOSPITAL DIRECTORY

User Name:
Password:
Home | FREE Search | FAQs | Sources | Updates | Order Info | Login

Individual Hospital Statistics for New York

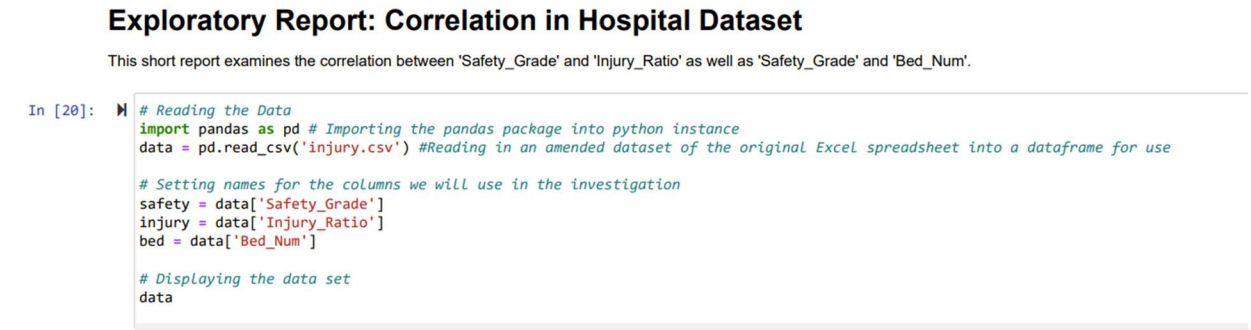
Statistics for non-federal, short-term, acute care hospitals.
Data are based on each hospital's most recent cost report and other sources / Definitions

Hospital Name	City	Staffed Beds	Total Discharges	Patient Days	Gross Patient Revenue ($000)
Long Island Community Hospital	Patchogue	184	8,389	53,998	$1,208,779
A.O. Fox Hospital	Oneonta	191	1,700	7,364	$167,284
Adirondack Medical Center - Sarnac Lake	Saranac Lake	155	1,871	7,234	$288,726
Albany Medical Center	Albany	794	35,219	203,266	$3,361,287
Albany Stratton VA Medical Center	Albany	0	0	0	$0
Alice Hyde Medical Center	Malone	181	1,236	4,404	$215,155
ArchCare at Terence Cardinal Cooke Health Care Center	New York	615	9	20,546	$93,166
Arnot Ogden Medical Center	Elmira	309	8,185	41,979	$790,147
Auburn Community Hospital	Auburn	179	4,004	17,576	$276,940

Figure 5: Excerpt from the American Hospital Directory

3.3.4 Statistical Analysis

Before beginning our statistical analysis, we first standardized our data set to decrease data errors or variance. This was largely through eliminating hospitals in high-metropolitan areas, since we could not accurately determine which occupational injuries correlated to which hospital accurately due to the proximity of the hospitals. We then used python to create a code which looked for correlation between our data sets.



Exploratory Report: Correlation in Hospital Dataset

This short report examines the correlation between 'Safety_Grade' and 'Injury_Ratio' as well as 'Safety_Grade' and 'Bed_Num'.

In [20]:

```
# Reading the Data
import pandas as pd # Importing the pandas package into python instance
data = pd.read_csv('injury.csv') #Reading in an amended dataset of the original Excel spreadsheet into a dataframe for use

# Setting names for the columns we will use in the investigation
safety = data['Safety_Grade']
injury = data['Injury_Ratio']
bed = data['Bed_Num']

# Displaying the data set
data
```

Figure 6: Code used for statistical analysis to indicate correlation

3.4 Limitations

There are a few limitations to our approach to studying the culture of safety at New York Hospitals. To begin with, the culture of safety as a concept is an extremely complex and dynamic one that is difficult to measure. We decided to use patient outcomes to decipher it, but this excluded many aspects like policies or leadership that these hospitals may have but are not seen within the provided Medicare website metrics. Furthermore, all our data is online information with little context provided. We assumed the definitions of the columns in the NY workers compensation claims website. Next, we assumed that people who live in a 10-mile radius of the hospital would go to those specific hospitals.

Chapter 4: Results

We were unable to confirm our hypothesis that patient safety and occupational hazards were positively correlated. Figure 7 demonstrates bed/injury/population versus safety grade. The y-axis demonstrates the injury count standardized by the number of beds and the population in the given zip codes of the hospitals. This is a standardized metric enabling direct comparison between all the hospitals. If these standardizations did not occur, then larger hospitals would be compared with smaller hospitals, and conclusions would not be able to be made. The x-axis demonstrates the patient safety grade. The metric reflects the patient safety practices among the 25 hospitals studied using the grading described in Table 1. The graph demonstrates that their relationship is horizontal, meaning that there is no change or no correlation.

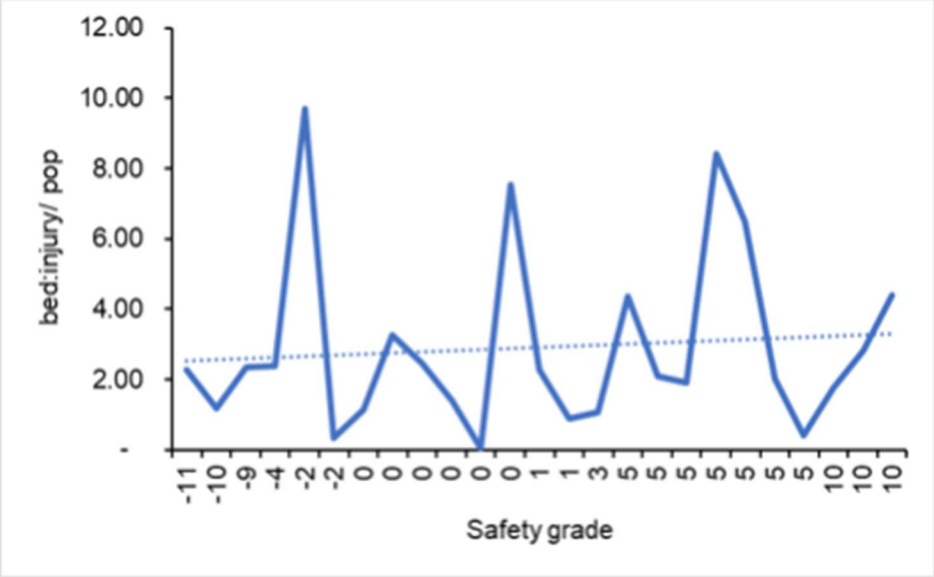


Figure 7: Comparison of safety grade to occupational injuries

Due to lack of positive slope, we can determine there is no correlation between safety grade and injury count, our hypothesis was not proven. While we were unable to find a positive correlation between patient safety and occupational hazard count, we identified a positive correlation between patient safety and the hospital size. Figure 8 has the safety grade on the y-axis while the number of beds is on the x-axis to demonstrate their correlation. The positive relation between these two variables was found to be statistically significant. These values, through the code demonstrated in Figure 9, had a strong correlation.

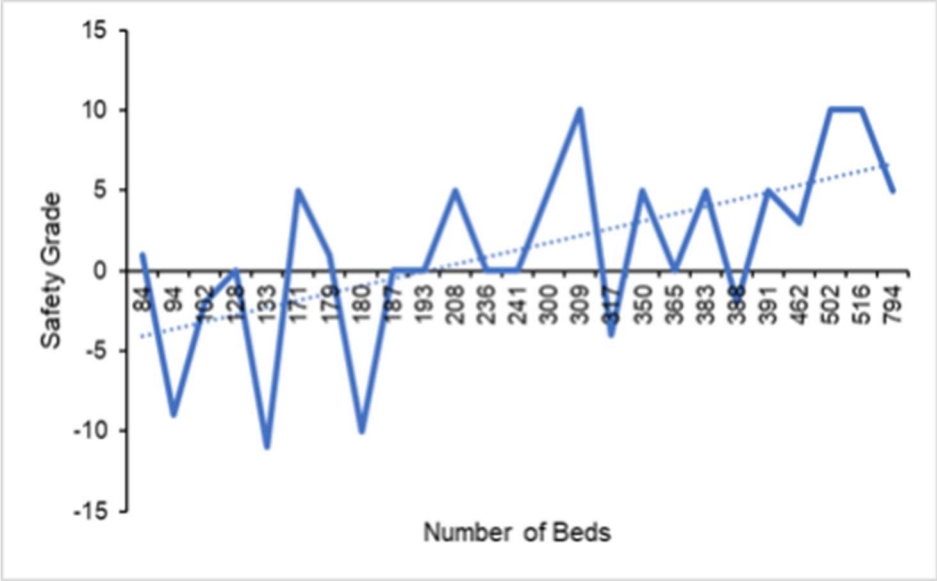


Figure 8: Safety grade is positively correlated with the sizes of the NY hospitals.

As the number of beds in the NY hospitals increases, the safety grade increases as well. In other words, the larger the hospitals are, the better patient outcomes they tend to have. This was a statistically significant finding.

Correlation between # of Beds and Safety Grade

```
In [23]: bed_to_safety = bed.corr(safety)
         bed_to_safety

Out[23]: 0.6876088751789826
```

0.7 indicates that there is strong positive correlation between the # of beds and the safety grade.

```
In [ ]:
```

Figure 9: Code used to determine correlation of initial factors.

A strong correlation was indicated between the number of beds and the safety grade determined from the Medicare website.

In comparison, Figure 10 demonstrates safety grade versus robustness, a variable that is indicative of the hospital's quality of reporting data. This illustrates a positive correlation between the two suggesting that the better safety practices a hospital has, note-taking, policy, or strong leadership as examples, then the better patient outcomes they will have. More specifically, based on data robustness, hospitals which reported their patient outcomes were more likely to have less occupational accidents. Together, this suggests that larger hospitals were more likely to report data, indicating a developed culture of safety.

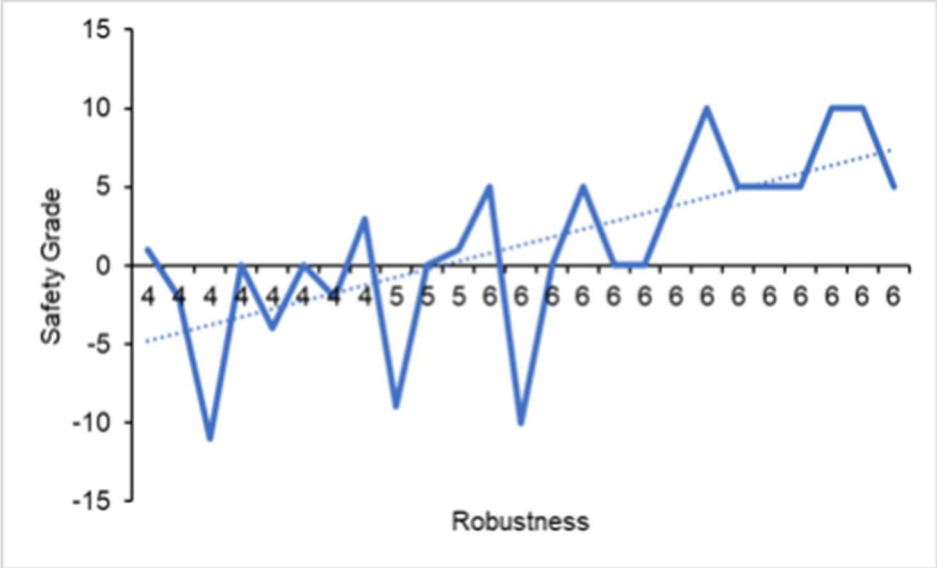


Figure 10: Patient outcomes become better with improved safety practices.

Safety grade versus robustness elucidates a positive correlation. This means that as robustness, or the ability of hospitals to report their data, improves, then their safety grade improves as well.

Chapter 5: Discussion

The focus of this project was to establish whether there was a relationship between the culture of safety within the workplace and overall patient safety. Through a two-step approach, we created a dataset that represents both the occupational safety, as well as patient safety of hospitals located in New York state. From there, we were able to paint a picture of the safety culture of each hospital, allowing a comparison between their occupational and patient safety.
Overall, we found that our hypothesis was unable to be proven based on the data we collected. Based on the data sets, there was no direct correlation between patient and occupational safety, however, we found a correlation between the culture of safety and hospital size. There was a positive correlation between the hospital size and patient safety.

When standardized, we found that hospitals that reported their data were more likely to have were more likely increased safety grades. In the Medicare data that the patient outcomes were based on, some of the values did not contain records of patient outcomes indicating hospitals were not reporting on common procedures. This was indicative that increased quality of safety in hospital work environments is directly correlated to the hospital's quality of reporting their quality of patient care. Essentially, if hospitals put more emphasis on reporting their data, they are more likely to have an increased culture of safety. Plausible explanations as to why this could be occurring in some hospitals and not others could be access to monetary resources, effective management, and increased worker interactions. These factors were not included in our data set, as they are qualitative factors, and our study focused mainly on quantitatively accessing a culture of safety. Despite not being included in our studies, these qualitative factors are very likely to be impacting safety scores as they also make a significant impact. Overall, our data was indicative that larger hospitals were more likely to report data on patient care, which was ultimately correlated to a higher level of safety in these institutions.

Further research can be focused on understanding why this occurrence occurs. Previous studies, such as ones done by the ECRI, have indicated how to create an effective culture of safety, as well as policies that can be put in place to improve it. To determine what factors are decreasing the culture of safety in smaller hospitals and increasing it in larger hospitals, further research can compare qualitative factors, such as policies or access to resources, to determine correlation. From there, further recommendations can be created to help increase culture of safety, with a focus increasing a culture of safety in small hospitals and maintaining them in larger hospitals.

In terms of our own research, the lessons learned from assessing the culture of safety in hospital environments can also be applied to laboratory environments. As biochemistry students who work consistently in research laboratories, understanding how to create an effective culture of safety is important for maintaining both our safety, the safety of others, and success in our research endeavors. Maintaining a culture of safety in a laboratory environment is like that of a

medical environment. It's important to provide a quantitative assessment of accidents that occur and identify sources of occupational hazards. In doing so, one can maintain a culture of safety, ensuring the quality of life for researchers and overall research. Taken together, "you are what you measure," is the main takeaway and one to bring in all future workplace environments.

Appendix

Appendix A: Summary of terms and definitions utilized

Term	Definition
Safety grade	A numerical value which represents hospital's ability to meet national standards (in reference to the Medicare data)
Injury count	The number of occupational injuries recorded within a 10-mile radius of the hospitals examined
Population	The number that appeared from a Google search "zip code," and "population."
Number of beds	The number of staffed beds according to the American Hospital Directory
Zip codes	These were used to combine the injury count and safety grades. The zip codes within a 10-mile radius of the hospitals were determined. Subsequently, the number of injuries were counted in each zip code and overlayed with the safety grades.
Measurement robustness	Measure of the hospital's capacity to report their data. It is identified by the number of Medicare categories for which the hospital reports complete data.

Appendix B: The common procedures defined in the Medicare website (Medicare.gov, 2022)

Criteria	Definition
Rate of complications for hip/knee replacement patients	Complications included in this measure are: infection, heart attack, pneumonia, wounds that split open or bleed after surgery, serious blood clots, replacement hip/knee joints that don't work, and death (Medicare.gov, 2022).
Serious complications	Complications may be a sign of poorer quality hospital care (Medicare.gov, 2022).
Deaths among patients with serious treatable complications after surgery	Refers to surgical patients who died after developing serious complications that could have been treated (Medicare.gov, 2022).
Central line-associated blood stream infections (CLABSI) in ICUs and select wards	A central line is a narrow tube inserted into a large blood vessel. When inserted incorrectly or not kept clean, central lines can become an easy

	way for germs to enter the body and cause serious infections, CLABSIs, in the blood. Include intensive care units (ICUs), neonatal intensive care units (NICUs), and adult and pediatric medical, surgical and medical/surgical wards (Medicare.gov, 2022).
Catheter-associated urinary tract infections (CAUTI) in ICU's and select wards	A catheter is a drainage tube inserted into a patient's bladder through the urethra and left in place to collect urine. When put incorrectly, kept clean, or when left in place for long periods of time, catheters can become an easy way for germs to enter the body and cause serious infections in the urinary Include ICUs and adult and pediatric medical, surgical, and medical/surgical wards (Medicare.gov, 2022).
Surgical site infections (SSI) from colon surgery or from abnormal hysterectomy	Compares the number of surgical site infections from specific types of operative procedures conducted at a hospital to a national benchmark (Medicare.gov, 2022)
Methicillin-resistant *Staphylococcus Aureus* (MRSA) blood infections	MRSA is a type of bacteria that is resistant to certain antibiotics. Hospital staff can prevent MRSA from being transmitted to patients by taking certain precautions, like washing hands; using protecting gloves and gowns; sterilizing equipment between patients etc… (Medicare.gov, 2022).
Clostridium difficile (*C. diff*) intestinal infections	*C. diff* is a type of bacteria that causes inflammation of the colon. Hospital staff can prevent *C. diff* in the same way they can prevent MRSA (Medicare.gov, 2022).

References

American Hospital Directory - Individual Hospital statistics for New York. (n.d.). Retrieved April 26, 2022, from https://www.ahd.com/states/hospital_NY.html

Department of Labor Logo United States department of Labor. Safety Management - Hazard Identification and Assessment | Occupational Safety and Health Administration. (n.d.). Retrieved April 20, 2022, from https://www.osha.gov/safety-management/hazard-Identification

Sisk, T. (2021, June 22). *A lasting legacy: Dupont, C8 contamination and the community of Parkersburg left to grapple with the consequences*. EHN. Retrieved April 20, 2022, from https://www.ehn.org/dupont-c8-parkersburg-2644262065/particle-7

Rich, N. (2016, January 6). *The lawyer who became Dupont's worst nightmare*. The New York Times. Retrieved April 20, 2022, from https://www.nytimes.com/2016/01/10/magazine/the-lawyer-who-became-duponts-worst-nightmare.html

Jachuck, S. J., Bound, C. L., Steel, J., & Blain, P. G. (1989, July 1). *Occupational hazard in hospital staff exposed to 2 per cent glutaraldehyde in an Endoscopy Unit*. OUP Academic. Retrieved April 20, 2022, from https://academic.oup.com/occmed/article-abstract/39/2/69/1355873

C E B. (1985). Occupational hazards to hospital personnel. *Journal of Occupational and Environmental Medicine*, *27*(11), 850. https://doi.org/10.1097/00043764-198511000-00021

Trinkoff, A. (2008). New resource for patient safety and quality. *Advances in Neonatal Care*, *8*(4), 193. https://doi.org/10.1097/01.anc.0000333700.86195.e5

Lawson, C. C., Rocheleau, C. M., Whelan, E. A., Lividoti Hibert, E. N., Grajewski, B., Spiegelman, D., & Rich-Edwards, J. W. (2013). Occupational exposures among nurses and risk of spontaneous abortion. *Obstetric Anesthesia Digest*, *33*(1), 36–37. https://doi.org/10.1097/01.aoa.0000426099.31929.92

Triolo, P. K. (1989). Occupational Health Hazards of Hospital Staff Nurses Part II. *AAOHN Journal*, *37*(7), 274–279. https://doi.org/10.1177/216507998903700704

Fleisher, L. A., Schreiber, M., Cardo, D., & Srinivasan, A. (2022). Health Care Safety during the pandemic and beyond — building a system that ensures resilience. *New England Journal of Medicine*, *386*(7), 609–611. https://doi.org/10.1056/nejmp2118285

ECRI. Culture of safety: an overview. Health Syst Risk Manage 2019 Jun 14.

Vogus, T., Weick, K. E., & Sutcliffe, K. M. (2010). Doing no harm: Enabling, enacting, and elaborating a culture of Safety in Health Care. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.1904620

Han, Y., Kim, J.-S., & Seo, Y. (2020). Cross-Sectional Study on Patient Safety Culture, Patient Safetyb Competency, and Adverse Events. *Western Journal of Nursing Research*, *42*(1), 32–40. https://doi.org/10.1177/0193945919838990

Weaver, S. J., Lubomksi, L. H., Wilson, R. F., Pfoh, E. R., Martinez, K. A., & Dy, S. M. (2013). Promoting a culture of safety as a patient safety strategy. *Annals of Internal Medicine*, *158*(5_Part_2), 369. https://doi.org/10.7326/0003-4819-158-5-201303051-00002

Guldenmund, F. W. (2000). The nature of safety culture: A review of theory and research. *Safety Science*, *34*(1-3), 215–257. https://doi.org/10.1016/s0925-7535(00)00014-x

Lingard, H., & Holmes, N. (n.d.). *Understandings of occupational health and safety risk control in small business construction firms: Barriers to implementing technological controls*. Taylor & Francis. Retrieved April 20, 2022, from https://www.tandfonline.com/doi/abs/10.1080/01446190010002570

Zoller, H. M. (2009). The social construction of occupational health and safety: Barriers to environmental-labor health coalitions. *NEW SOLUTIONS: A Journal of Environmental and Occupational Health Policy*, *19*(3), 289–314. https://doi.org/10.2190/ns.19.3.b

da Silva, S. L., & Amaral, F. G. (2019). Critical factors of success and barriers to the implementation of occupational health and Safety Management Systems: A systematic review of literature. *Safety Science*, *117*, 123–132. https://doi.org/10.1016/j.ssci.2019.03.026

Medicare.gov. (22 January,2022). Retrieved April 26, 2022, from https://www.medicare.gov/care-compare/all-results?searchType=Hospital&page=1&state=NY&emergencyServices=true&hospitalType=Acute+Care+Hospitals&sort=alpha&returnAllResults=true

Qi, J. (2019, January 19). *U.S. ZIP code query: United States ZIP code 5 plus 4*. U.S. ZIP Code 5 Plus 4. Retrieved April 26, 2022, from https://zip.postcodebase.com/

York, S. of N. (2021, July 1). *NYS Assembled Workers' Compensation claims*. Kaggle. Retrieved April 26, 2022, from https://www.kaggle.com/new-york-state/nys-assembled-workers'-compensation-claims
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7531934/