NEURAL EFFECTS OF ESTROGEN ON MITOCHONDRIAL LIPIDS AND CARDIOLIPIN LEVELS AND DISTRIBUTION

by

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DEDICATION

I dedicate this work to those studying and fighting neurodegeneration. May we provide and maintain hope.

Also, to those with a red line under their name in Microsoft Word.

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There are several people I would like to thank starting with:

My mentors over the years: Dr. Nadine Kabbani, Dr. Laryssa Huryn, and Dr. Oliver Kerscher. Their unfailing encouragement and reassurance, as well as challenges in work, have helped me to grow as both a person and a scientist, and helped to solidify my passion for studying neurodegenerative diseases.

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Finally to Lucy the cat, whose eyes never left the page while I typed…at least until she fell asleep.

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LIST OF ABBREVIATIONS

Adenosine triphosphate ..... ATP
Amyloid precursor protein ..... APP
Cardiolipin CL
Central nervous system ..... CNS
Estradiol E2
Inner mitochondrial membrane ..... IMM
Nonyl-acridine orange ..... NAO
Outer mitochondrial membrane ..... OMM
Phosphate buffered saline ..... PBS
Reactive oxygen species ..... ROS

ABSTRACT

NEURAL EFFECTS OF ESTROGEN ON MITOCHONDRIAL LIPIDS AND CARDIOLIPIN LEVELS AND DISTRIBUTION

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George Mason University, 2023

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A variety of lipid classes play important roles in neuronal structure and function. One of these lipids is cardiolipin, a phospholipid exclusive to the mitochondria and important to processes such as mitophagy and apoptosis through its differential oxidation. Estradiol (E2), a steroid sex hormone, is known to exert antioxidant effects in various cell types. I will establish a baseline lipidome in a line of neural cells and use E2-treatment conditions to determine if estrogen antioxidant effects are mediated through lipids, with a particular focus on cardiolipin. The results could contribute to understanding how hormonal changes can impact neural cell health and neurodegeneration.

CHAPTER ONE: LIPIDS IN NEURODEGENERATION

INTRODUCTION

Various types of lipids can be found throughout the central nervous system (CNS), accounting for about half of the brain's dry weight. The primary species of lipids within the CNS are phospholipids (~50%), sphingolipids (~40%), cholesterol (~10%), as well as trace amounts of triglycerides (Sastry, 1985). CNS lipids perform three primary functions: 1) structural components of neural cell membranes; 2) long-term energy storehouses; 3) signaling molecules that can bind and modulate the activity of various receptors, ion channels, and second messenger molecules (Mutlu et al., 2021). They are also known to act as messengers between cells participating in signaling for inflammation and homeostatic regulation (López-Vales & David, 2019). Lipids are particularly abundant in the brain, which has the second highest lipid content in the human body after adipose tissues. Regarding individual neurons, lipids make up about 50–60% of the cell membrane. Among the most abundant lipid species identified in the brain are cholesterol, phospholipids including phosphatidylcholine and phosphatidylethanolamine, and sphingolipids (Yoon et al., 2022).

Brain lipid composition may provide not just important data about how a healthy brain functions, but also about changes that occur with aging and neurodegeneration. Two studies in the late 1950s found that during the first twenty years of life, there was an

increase of total lipids in the brain, but that later in adulthood and advanced age, there was a progressive decrease in total lipids, and especially seen in certain species (Burger & Seidel, 1958; Naudí et al., 2015; Rouser & Yamamoto, 1968). Alzheimer's disease, characterized by the miscleavage of amyloid precursor protein (APP), an increase in oxidized or inflammatory lipid species may shift the cleaving process of APP from nonamyloidogenic to the amyloidogenic pathway, favoring the γ-secretase cleavage (Chew et al., 2020). Parkinson's disease may also be characterized by an excess of lipids binding to α-synuclein, causing a build-up in the system and eventually turning to Lewy bodies (Fanning et al., 2020). This indicates that lipid alterations are implicated in actively shifting a healthy system to a diseased one. In addition, high levels of ceramides have been found in the cerebrospinal fluid of patients with multiple sclerosis, while increased levels of ceramides and glucosylceramides have been observed in patients with amyotrophic lateral sclerosis, meaning that lipid alterations have been found in other neurodegenerative diseases, begging the question of whether those might be underlying causes (Saito et al., 2021).

Besides lipid alterations, another underlying molecular cause of neurodegeneration is mitochondrial dysfunction. Mitochondria are responsible for the energy upkeep of the cell, providing the necessary energy in the form of adenosine triphosphate (ATP) and helping to maintain cellular homeostasis by getting rid of reactive oxygen species (ROS). The activity and maintenance of nervous system tissue is highly energy demanding, with the brain using ~ 20% of the body's total energy expenditure (Camandola & Mattson, 2017). Neurons utilize up to 80% of that energy and rely on the

metabolism of glucose, lactate, and ketone bodies, as well as mitochondrial oxidative phosphorylation to generate energy (Camandola & Mattson, 2017; Harris et al., 2012; Hyder et al., 2013; Pfanner et al., 2019). Due to this high energy demand, neurons appear to have more mitochondria than most other types of cells, which enables neuronal energy demands to be met via mitochondrial energy creating processes such as cellular respiration and oxidative phosphorylation (Rango & Bresolin, 2018). To maximize the production of ATP, the mitochondria is double-membraned and has many cristae to increase the surface area (Lu & Claypool, 2015; Paradies et al., 2019). Each mitochondrion contains an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM), with an intermembrane space separating the two. Both the OMM and IMM differ in lipid composition and the distribution of phospholipids (Falabella et al., 2021; Lu & Claypool, 2015; Paradies et al., 2019). Changes to the composition of these phospholipids within the mitochondrial membranes affect both the structural integrity and the energy generating properties of the mitochondria such as electron transport chain and oxidative phosphorylation.

One of the signature phospholipids of the inner mitochondrial membrane is cardiolipin (CL), making up nearly 20% of the total mitochondrial phospholipids (Paradies et al., 2019). Unlike other phospholipids, CL has four acyl chains connected to two phosphate groups linked to a glycerol head. These chains give CL a conical shape, enabling it to contribute to the curvature of the inner cristae (Gasanoff et al., 2021; Paradies et al., 2019). Under physiological conditions, CL is absent from the OMM and present in the cristae of the IMM where it faces the intermembrane space, and

participates in important functions in energy metabolism (Falabella et al., 2021). CL is heavily prone to peroxidation for several reasons. One, its high composition of acyl chains can undergo nonenzymatic autoxidation to form a peroxide dimer that can create alkoxyl radicals (Su et al., 2019). Another is its physical association with cytochrome c, causing it to form a complex that has peroxidase activity targeting the acyl chains of CL and thus increasing the amount of oxidized CL (Bajwa et al., 2019; Kurokin et al., 2021; Pizzuto & Pelegrin, 2020). Cytochrome c has a lower affinity toward oxidized CL, causing oxidized CL to detach from the IMM and become externalized. This externalization of CL signals apoptosis either through interaction with pro-apoptotic factors such as Bcl-2 family proteins or caspase 8 (Falabella et al., 2021). Third, the proximity of CL in the IMM to the complexes involved in the electron transport chain can lead to uncontrolled oxidation, which can induce conformational changes of the IMM. These potential structural changes of the IMM impacts the cellular energy processes, which may compromise the mitochondrion (Bajwa et al., 2019; Kurokin et al., 2021; Pizzuto & Pelegrin, 2020). Changes like this can and do cause pathogenesis of certain diseases (Falabella et al., 2021).

Estrogens are a group of steroid hormones including estrone, estradiol (E2), estriol, and estetrol (Fuentes & Silveyra, 2019). The most physiologically common one in humans is E2, from which estrone and estriol are derived and which has the highest affinity for intracellular estrogen receptors (Russell et al., 2019). Estrogen is known to play a role in reward and feeding behavior and can modulate cognitive processes such as learning (Russell et al., 2019). Within mitochondria specifically, estrogen regulates many

enzymes involved in cellular respiration and oxidative phosphorylation, and has been shown to exert antioxidant effects (Klinge, 2017; Prokai et al., 2003; Russell et al., 2019; Simpkins et al., 2008; Ventura-Clapier et al., 2019). One group even reported that 17-beta estradiol and estrone reduced lipid peroxidation in selected rat and human neural cell homogenates (Vedder et al., 1999).

It is a sudden loss of estrogen during menopause however that leads scientists to believe that this abrupt decrease in estrogen might be the reason women are more susceptible to neurodegeneration, Alzheimer's disease, or general dementia than men (Rettberg et al., 2014; Shaw, 2021). The precise reason as to why or how is unknown, but estrogen, through its estrogen receptors, has been shown to influence the regulation of aerobic respiration and glucose transport, indicating that the sudden drop in estrogen would leave menopausal women unable to meet their body's energy demands (Arnold & Beyer, 2009; Del Río et al., 2018; Rettberg et al., 2014; Shaw, 2021). Because CL plays such as a pivotal role in the mitochondria, we wanted to examine the effects of E2 withdrawal on cardiolipin by looking at CL levels in human neuroblastoma SH-SY5Y cells treated with E2.

In this study, we examined estrogen alterations to cardiolipin fluorescence separately in both non-differentiated and differentiated SH-SY5Y cells, which are an established model system for neurodegenerative diseases (Kovalevich & Langford, 2013). Our results demonstrate the role of estradiol in altering levels of cardiolipin, potentially contributing to why women are more susceptible to cognitive decline and dementia.

MATERIALS AND METHODS

Cell Culture and Estrogen Treatment

Non-differentiated SH-SY5Y cells (American Type Culture Collection, CRL-2266) seeded on a 24-well glass bottom plate coated with 100 µg/ml poly-D-lysine (Millipore, A-003-E) were incubated in a culture media solution of DMEM (Gibco, 11965-092,), 1% penstrep (Quality Biological, 120-095-721), and 10% fetal bovine serum (FBS, VWR, 97068-085) at 37°C in 5% carbon dioxide.

The cells were treated as follows: a third of the wells received no treatment, only media; another third received 100nM **β**-estradiol (E2, Sigma-Aldrich, E2758) for three days, with another treatment of E2 after those three days were up; the remaining third was treated with 100 nM concentration of E2, then only untreated media. To create the 100 nm E2, 1 mL media was mixed with 1 µL of 20mM E2/EtOH to dilute it to 20 µM E2. I then used 35 µL of that solution in 3.5 mL of media to ensure proper concentration.

Differentiated SH-SY5Y cells (American Type Culture Collection, CRL-2266) seeded on a 24-well glass bottom plate coated with 100 µg/ml poly-D-lysine (Millipore, A-003-E) were incubated in a culture media solution of DMEM (Gibco, 11965-092,), 100 µL N_2, 100 µL glutamax, 5 µL retinoic acid, and 3% fetal bovine serum (FBS, VWR, 97068-085) at 37°C in 5% carbon dioxide. These cells were treated as follows: half of the wells received no treatment, only media, and the other half received 100nM **β**-estradiol (E2, Sigma-Aldrich, E2758) for three days, with another treatment of E2 after those three days were up. Again, to create the 100 nm E2, 1 mL media was mixed with 1 µL of

20mM E2/EtOH to dilute it to 20 µM E2. I then used 35 µL of that solution in 3.5 mL of media to ensure proper concentration.

Cardiolipin Labeling with Nonyl Acridine Orange and Phalloidin Staining

Seventy-two hours after the 100% media change, staining was done using nonyl acridine orange (NAO) and phalloidin. I made a 5 µM NAO solution in media, and removed 50% of the media from each well. I treated the cells with 5 µM NAO solution in media for 30 minutes, during which it was incubated at 37°C in 5% carbon dioxide. After removal of the NAO, the cells were then washed with phosphate buffered saline (PBS). Cells were then fixed in a 0.3% solution of glutaraldehyde (1X PBS with 80 mM PIPES, 1m mM $MgCl_2$, 5 mM EDTA, and 0.3% glutaraldehyde), and labeled with rhodamine tagged phalloidin. There was no difference in labeling and staining between the differentiated and non-differentiated cells.

Confocal Microscopy & ImageJ

Cells from each well were then imaged using a confocal microscope, Zeiss Axio Observer LSM 800, ensuring that each was on the same laser intensity setting to keep the fluorescence constant. Emission and excitation spectra for the NAO was at 509 nanometers and 488 nanometers, respectively, and 572 and 545 nanometers for phalloidin, respectively. At least 5 separate images were taken from each well. CL levels were then measured by analyzing the fluorescence of the images in ImageJ. Cells were manually outlined using the phalloidin stain and with the "measure" function in ImageJ, fluorescence of the NAO stain and the area of the cell were taken down. Raw integrated density fluorescence measures were normalized to the outlined area and then either a t-

test or a one-way ANOVAs with Tukey's HSD for multiple corrections were completed using RStudio. Any differences with $p<0.05$ were considered statistically significant.

RESULTS

Effect of E2 Treatment on Non-Differentiated SH-SY5Y Cells

We first examined the effects of estrogen on the fluorescence of cardiolipin using nonyl-acridine orange (NAO) in human non-differentiated SH-SY5Y neuroblastoma cells. We compared between the presence of estrogen in E2+ cells that had been treated with 100nM E2 for six days, estrogen withdrawal in E2# cells that were treated with E2 for three days and then withdrawn and grown for a further three days, and a no estrogen control condition labeled as E2- cells. Measuring the NAO signal intensity showed that cardiolipin increases within E2# cells when compared both to E2- ($p = 0.0022$) and E2+ ($p = 0.029$) cells. We did not detect a difference in cardiolipin levels between E2- and E2+ cells ($p = 0.678$).

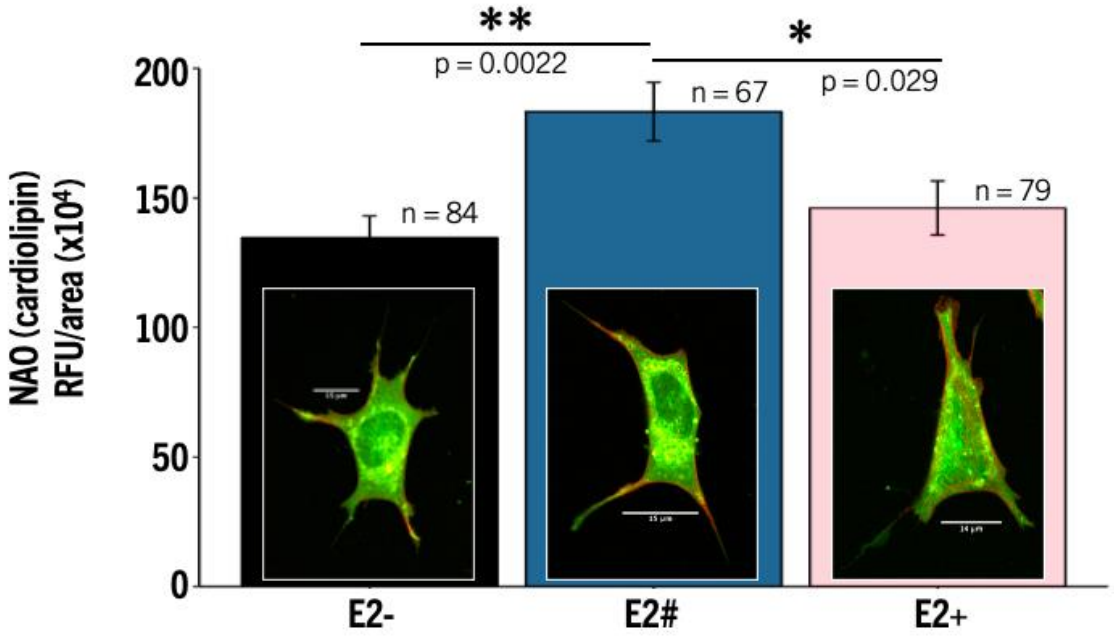

Figure 1. Effect of E2 Treatment on Non-Differentiated SH-SY5Y Cells.

Effect of E2 Treatment on Differentiated SH-SY5Y Cells

We then examined the effects of estradiol on the fluorescence of cardiolipin in differentiated SH-SY5Y cells. We had groups set up with either the presence of estrogen in E2+ cells that had been treated with 100nM E2 for six days and a no estrogen control condition labeled as E2- cells. Measuring the NAO signal intensity showed that cardiolipin increases in E2- cells when compared to E2+ ($p = 0.0041$) cells.

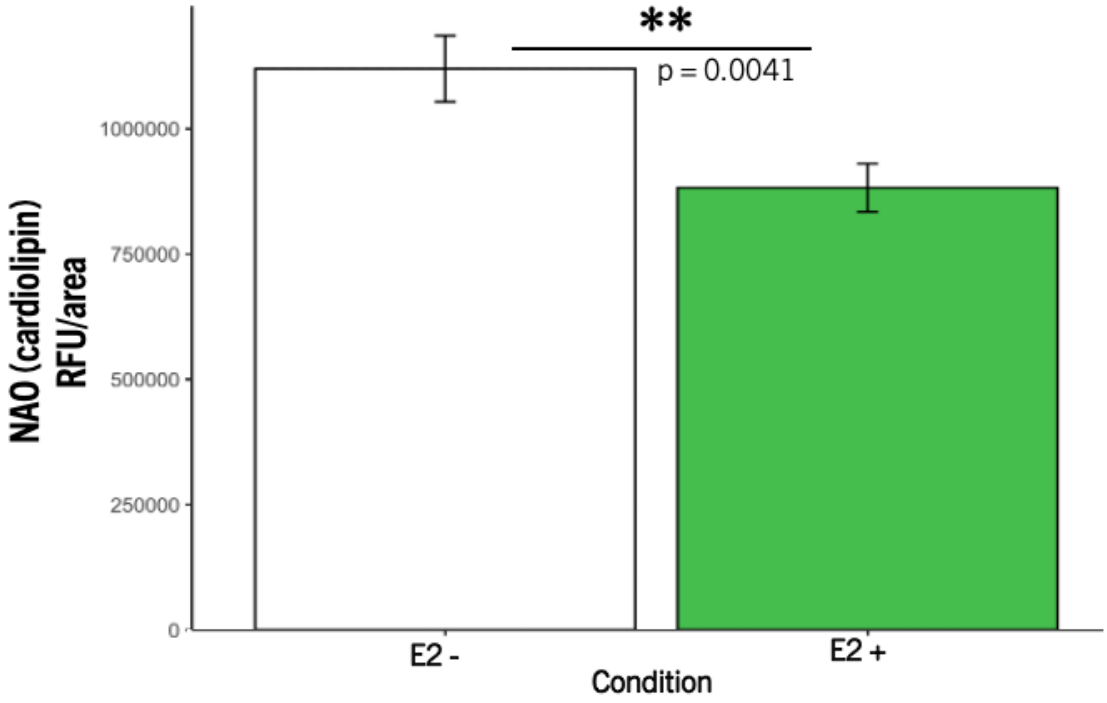

Figure 2. Effect of E2 Treatment on Differentiated SH-SY5Y Cells.

Effect of E2 Treatment on Neurite Growth in Differentiated SH-SY5Y Cells

We then examined the effects of estradiol on the neurite length of differentiated SH-SY5Y cells. We had groups set up with either the presence of estrogen in E2+ cells that had been treated with 100nM E2 for six days and a no estrogen control condition labeled as E2- cells. Taking the sum of the neurite lengths in each group, we measured a total length of 3454.51μm in E2- (n = 51) and 2741.634 μm in E2+ (n = 45), indicating a more neurite growth in E2- (p = 0.425).

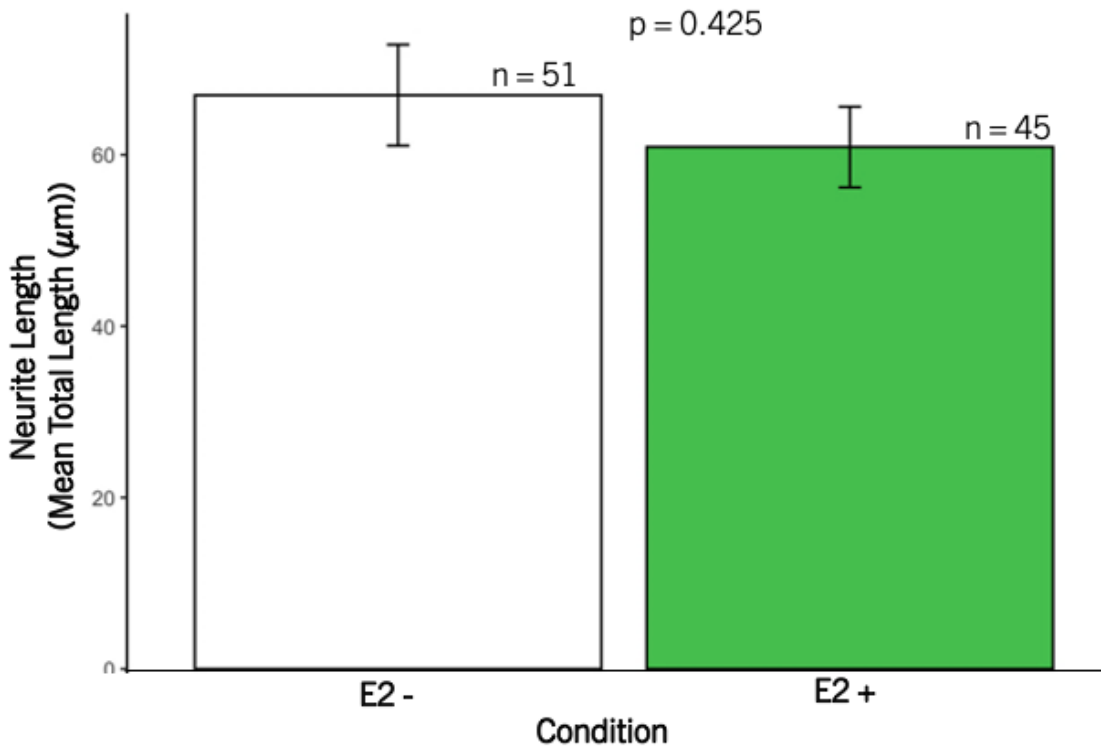

Figure 3. Effect of E2 Treatment on Neurite Length in SH-SY5Y Differentiated Cells

DISCUSSION

Evolutionary reasons for a sudden drop in estradiol at advanced age is debated, but it is believed to be a side effect of human longevity and meiotic arrest found only in humans and three species of whale (Huber & Fieder, 2018). This decrease in estrogen has been shown to increase cellular susceptibility to stress, resulting in higher production of reactive oxygen species and ultimately decreasing the ability of the mitochondria to respond to physiological energy demands (Klinge, 2017; Prokai et al., 2003; Russell et al., 2019; Simpkins et al., 2008; Ventura-Clapier et al., 2019). This estradiol-mediated susceptability might play a role through its actions, direct or indirect, on cardiolipin.

Estrogen is known to have a neuroprotective effect on mitochondria by impacting mitochondrial metabolism and by increasing cytochrome c in the cell (Grimm et al., 2012; Razmara et al., 2008). In disease, there can be an increase in mitochondria observed as the body adapts to an increased need for energy (Nunnari & Suomalainen, 2012). However, this does not necessarily mean that this demand will be met. In cancer, for instance, an increase in mitochondria for tumor growth naturally necessaitates an increase in cardiolipin. One study found that the cardiolipin measured in tumor cells contained major remodeling defects, leading to decreased electron transport chain function and ATP production (Kiebish et al., 2008), indicating that although an increase in cardiolipin levels is seen, there may not be a correlation to healthy cardiolipin or mitochondrial function. Our results, as seen in Figures [], indicate that estrogen does affect cardiolipin. In the three-condition set-up, one can see a statistically significant increase in levels of cardiolipin in the E2# setup and similarly, in the two condition set-up between E2- and E2+, one can see a significant increase in the E2- as compared to the E2+. We have shown that estrogen withdrawal has an effect on cardiolipin, potentially affecting mitochondrial function and demonstrating that an increase in cardiolipin might be representative of an at-risk state of disease.

CHAPTER TWO: BUILDING A LIPIDOME IN SH-SY5Y CELLS

INTRODUCTION

Lipids are a structurally diverse set of compounds chemically characterized by their solubility in organic solvents, and insolubility in polar solvents such as water or chloroform. Lipids are composed primarily of carbon and hydrogen, but may also contain oxygen, nitrogen, sulfur, and/or phosphorus. Lipids can be generally classified into: fatty acids, a carboxylic acid with a hydrocarbon tail; triacylglycerols, fatty acids with a glycerol head; cholesterol esters, fatty acids with a cholesterol head; phospholipids, fatty acids with a phosphate head; and sphingolipids, fatty acids with a sphingoid base (Fantini & Yahi, 2015; Mutlu et al., 2021).

Lipidomics refers to the large-scale study and analysis of lipid molecules or whole assemblies of lipids, called the lipidome. A typical lipidomics analysis comprises of: 1) sample preparation, in which one extracts the lipids out of their biological sample; 2) data acquisition, allowing one to simplify the complexity of the sample or tag specific functional groups of lipids; 3) data processing, examining the results one has obtained; and 4) data interpretation, analyzing and correlating the data against lipid databases or other datasets (Züllig et al., 2020). Due to the innate chemical complexity of lipid structures, the high diversity of lipid species, and the various challenges in lipid identification and analysis, lipidomics developed more slowly than other fields. Within

the past twenty years however, the output of publications involving lipidomics has increased dramatically, making it one of the fastest-growing scientific research fields (Züllig et al., 2020; Züllig & Köfeler, 2021).

Lipidomics is a young field however, and lipids and their functions in living systems are still understudied. Because there are no direct genetic methods to generate and manipulate lipids in cells, lipid biology has become largely dependent on chemical tools and analytical methods. Mass spectrometry remains the most popular method to identify lipids. This method has issues, including the ionization of neutral lipids, unknown fragmentation patterns of lipids, and because of the high diversity of molecular lipid species, there may be many potential mass spectral overlaps of lipid molecular ions and molecular adduct ions in spectra, among other problems (Murphy, 2018; Smith et al., 2014; Züllig & Köfeler, 2021). Despite being a young field, it is aiding in the development of new chemical techniques, such as new simulations to study molecular dynamics, improvements to mass spectrometric analysis, and new imaging methods (Murphy, 2018; Smith et al., 2014; Yang & Han, 2016; Züllig & Köfeler, 2021).

In this study, we examined estrogen alterations to whole-cell lipidome separately in non-differentiated SH-SY5Y cells. Our results demonstrate the role of estradiol in altering the lipidome, contributing to how a sudden loss of estrogen might alter lipid homeostasis and what the new lipidome would look like.

MATERIALS AND METHODS

Cell Culture

SH-SY5Y cells (American Type Culture Collection, CRL-2266) were grown in T75 flasks with non-differentiated media. Incubations and centrifugations were done at 4°C. Cells were scraped and after a 20 minute incubation at 4°C, cell lysates were centrifuged at 720 x g and the resulting supernatant was centrifuged again at 10,000 x g for 10 minutes to obtain a pellet.

Lipid Isolation & Extraction

Lipids were extracted using a 1:1:2 ratio of methanol, water, and chloroform, respectively as previously described by Bligh and Dyer (Bligh & Dyer, 1959). The pellet was briefly homogenized in water and methanol first, incubated on ice for an hour, and then the chloroform was added. The solution was then vortexed, spun down, and the bottom layer extracted. Two more washes were done using chloroform to ensure efficient extraction. The chloroform solvent was then evaporated under nitrogen.

Mass Spectrometry

The sample extracts were analyzed on a Sciex QTRAP 4500 mass spectrometer equipped with a Shimadzu Prominence UFLC XR System, whereas, it was separated on Phenomenex Kinetex 2.6 μm F5 100 A LC column, 100 X 2.1 mm, PN: OOD-4723-AN. The column temperature was kept at 40 ^{0}C. Solvent A was 60% LCMS grade water and 40% LCMS grade acetonitrile acidified with 0.1% formic acid and solvent B was 10% LCMS grade acetonitrile and 90% LCMS grade isopropanol acidified with 0.1% formic acid. The source temperature was kept at kept at 220 ^{0}C and the ion spray voltage

was 4500 volts. The flow rate was constant throughout the method and was set to 0.3 mL/min. The gradient was as the following:

Table 1. Flow Rate Gradient for the LCMS

Time (min)	A%	B%	Flow rate (ml/min)
0	100	0	0.3
2	100	0	0.3
20	0	100	0.3
25	0	100	0.3
25.1	100	0	0.3
30	100	0	0.3

The mass spectrometry method was set to both positive and negative MRM modes with a cycle time of 0.9950 second. All transitions had a dwell time of at least 7 milliseconds depending on the number of acquired transitions in each method. The collision energy was set to volts specific to each analyte. The entrance potential was set to 10 volts and the declustering potential was set to at least 40 volts. The collision cell exit potential was set to 14 volts (Cheema et al., 2020, 2021, 2022; Dissmore et al., 2021; Girgis et al., 2021; Hinzman, Jayatilake, et al., 2022; Hinzman, Singh, et al., 2022; Li et al., 2021, 2022). Data was analyzed using Analyst 1.7 and Sciex OS software for quantification.

RESULTS

Comparison of Chloroform and Isopropanol Methods Lipidomics

Comparing the two runs, we found more lipid species per group with the chloroform method. Of all the groups, we found the most species of

phosphatidylethanolamine, phosphatidylserine, sphingomyelin, phosphatidylcholine, and phosphatidylinositol. Cardiolipin had an equal number of species found between the two runs. It should be noted that these results are reflective of the types and number of species found in the sample, not necessarily of the abundance of the species.

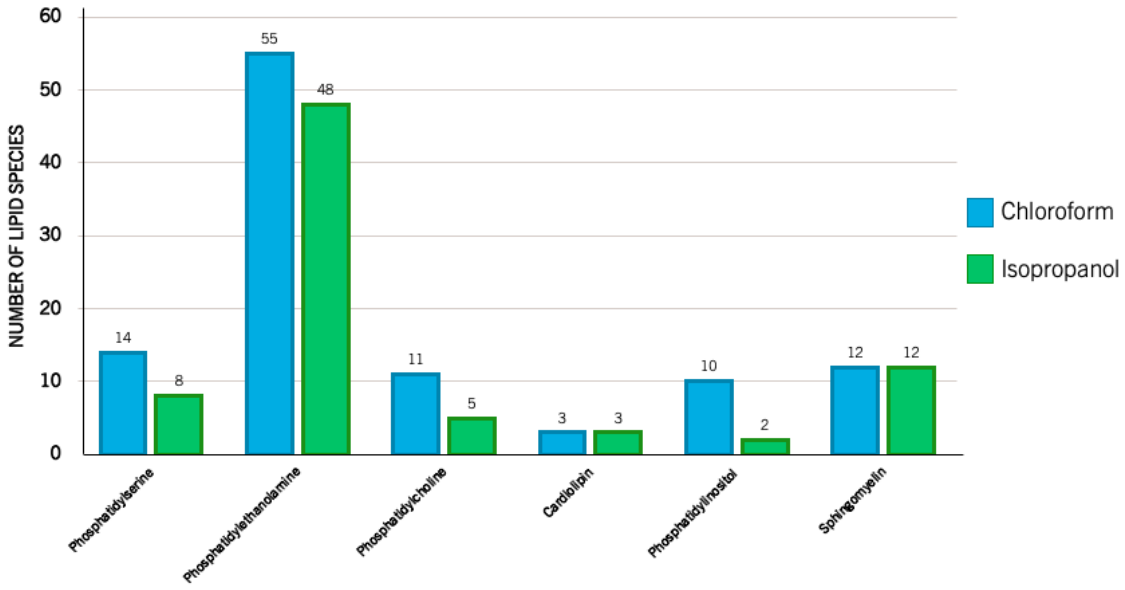

Figure 4. SH-SY5Y Whole Cell Lipidomics (Chloroform vs. Isopropanol)

Non-Differentiated SH-SY5Y Cells Lipidomics Results

We repeated the run, but this time only using the chloroform method. Of all the groups, we found the highest number of species of phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, and phosphatidylinositol, followed by others. Miscellaneous includes several species of ethanolamides, maresin conjugates in tissue regeneration 3 (MCTR3), and 5,15-diHETE. Again, it should be noted that these results are reflective of the types and number of species found in the sample, not necessarily of the abundance of the species.

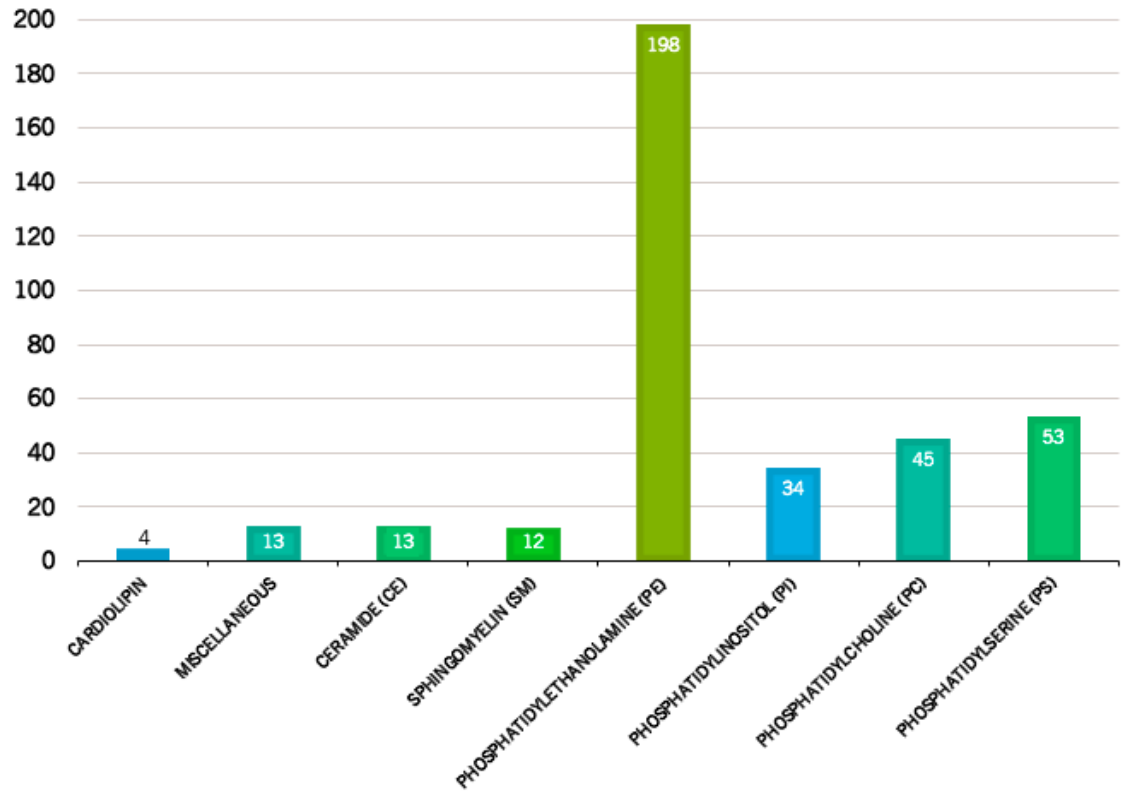

Figure 5. SH-SY5Y Whole Cell Lipidomics Second Run

Effect of E2 Treatment on Non-Differentiated SH-SY5Y Cells

Our data showed different peak area values for each species across the three conditions. We took the sum of the species for each group, and then took the ratio across the three conditions relative to the E2 minus group. We found that the E2 withdrawal conditions appear to show the most change for certain groups, while the no E2 and consistent E2 did not differ too much from each other. In cardiolipin and phosphatidylcholine, both mitochondrial lipids, this change is especially marked with E2 withdrawal showing an increase in both groups. This increase in cardiolipin in the withdrawal group also does match with our non-differentiated results from before, in which there was a statistically significant increase in the fluorescence of cardiolipin in the withdrawal group.

Conversely, we do see a decrease in ceramides for E2 withdrawals. The remaining ratios did not drastically differ from one another.

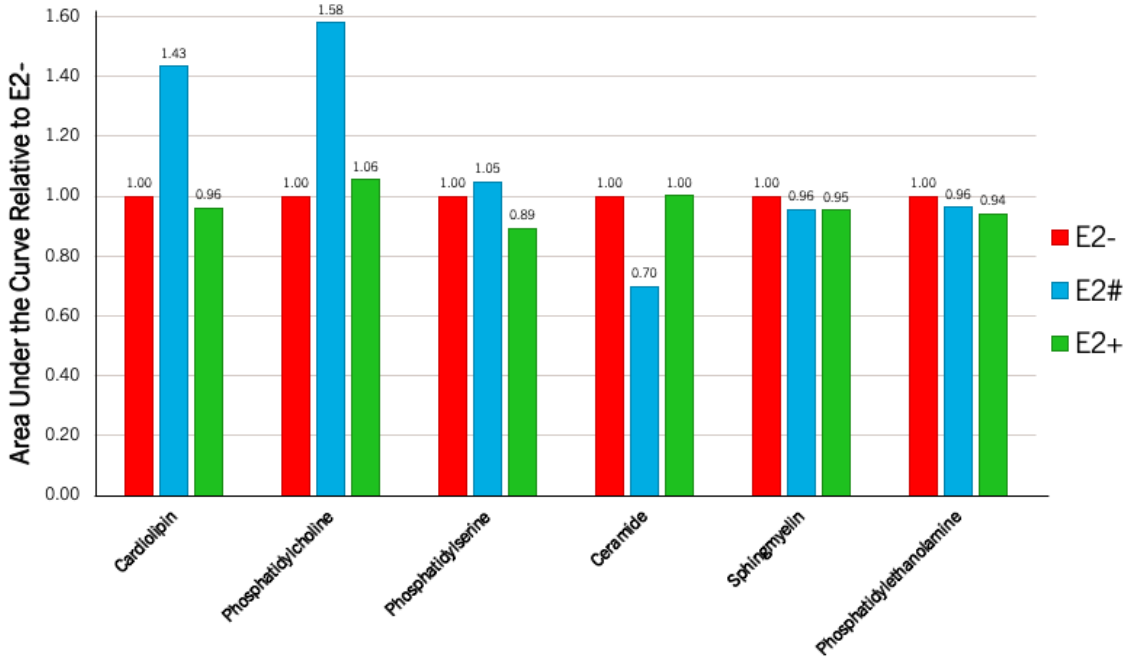

Figure 6. Effects of E2 Treatment on SH-SY5Y Whole Cell Lipidomics

DISCUSSION

Age-related decreases in estradiol has been known to affect the female lipidome. Menopause can reduce high-density lipoprotein cholesterol and increasing the total cholesterol, triglycerides, and low-density lipoprotein cholesterol (Reddy Kilim & Chandala, 2013). It has also been documented as decreasing mitochondrial function by decreasing the uptake of glucose, expression of genes required for bioenergetic demands, and mitochondrial respiration (Yin et al., 2015).

One study analyzing phospholipids in SH-SY5Y cells found that phosphatidylcholine was the most abundant phospholipid, with phosphatidylethanolamine and cardiolipin

following, respectively (Jakubec et al., 2019). However, these differences may also be due to differences in lipid extraction, as that study had used a mixture of dichloromethane and methanol, rather than chloroform and methanol. Cardiolipin, too, has its difficulties in mass spectrometry analysis as it is easily oxidized (Xu et al., 2018). To date however, there has not yet been any studies specifically looking for estrogen effects on lipids and whether or not this might be impacting mitochondrial function. Our study helps to start filling in that information. Our lipidomics results showing an increase in cardiolipin and phosphatidylcholine in E2# condition is interesting (Figure 6), as both being abundant in the mitochondria would indicate that one is witnessing a change in the mitochondrial lipidome. This could be further studied by doing a mitochondrial fraction, rather than a whole cell examination.

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