LOGOS

A Journal of Undergraduate Research

Volume 14

Fall 2021

ISSN 2153-1560 (print)
ISSN 2153-1579 (online)

LOGOS
Honors College
Missouri State University
901 South National Avenue
Springfield, MO 65897
honorscollege@missouristate.edu

Cover design by Diana Dudenhoeffer

Editorial Statement

Dear readers,

The *LOGOS* student and faculty editorial staff are pleased to introduce Volume 14, especially in light of the continuing hardships we've all faced for almost two years now. Another successful volume of *LOGOS* is here in spite of a continuing pandemic, and our authors have persisted in providing insight into the state of the world around us in their research. We applaud them for their work in this difficult time.

Last year, Volume 13 formed themes of social justice and grief independently of the pandemic, a reflection of what our authors were seeing take place around them. While considering the themes of Volume 14, we wanted to reflect the continuing state of the world in our cover design. Our copy editor and page designer, Diana Dudenhoeffer, presented us with the design that she felt best represented enduring a pandemic for multiple years. She set out to capture the continuing feeling of isolation and the hope of being able to safely adapt to the world as it is now. The red moving behind the letters in the title represents this transition from isolation to adaptation, and we wanted the repeating state of "isolation" to be a ghostly backdrop for the title. The red is repeated in the bright, fully opaque "adaptation," reflecting the necessity of focusing on hope for change. After two years of loss and illness, we hope this cover represents the darkness of that time, but also the hope for light in the future.

Since its inception in 2008, *LOGOS* has been committed to publishing high-quality, cutting-edge research, artwork, and creative writing produced by undergraduate students at Missouri State University. We are also committed to offering a safe and inclusive environment for students to engage in the vulnerable act of submitting their work for critique and scrutiny and experiencing the often intense world of academic publication for the first time. As a publication, we strive to introduce and amplify new voices to the conversation and creation of new knowledge, particularly those voices that may otherwise be silenced, whether by bureaucracy and red tape in the professional world or by systemic barriers that exclude minority students and professionals from sharing their perspectives.

With Volume 14, we reaffirm our commitment to our readers, our student body, and our student and faculty editors to continue providing a forum that challenges the status quo and expresses part of the human condition, invites underrepresented student voices into the ongoing academic con-

versation, showcases the best of what's happening at Missouri State University and provides low-risk, high-reward opportunities for students to improve their writing and learn more about publishing in their field. We hope you enjoy reading Volume 14 as much as the editorial board enjoyed putting it together.

Sincerely,

Emma M. Bishop
Managing Editor, 2021–2023

Dedication

We dedicate this volume of *LOGOS: A Journal of Undergraduate Research* to the past and present Missouri State University students, faculty, staff, and community members who have faced loss and illness in the past two years directly or indirectly resulting from COVID-19. Thank you for enduring the fight even during a continuing pandemic, and know that you are loved.

Faculty Advisory Board

John Chuchiak, IV, PhD: *Director,* Honors College; *Distinguished Professor,* Department of History, College of Humanities and Public Affairs
Tracy Dalton, MA: *Senior Instructor*, Department of English, Judith Enyeart Reynolds College of Arts and Letters
Joshua J. Smith, PhD: *Professor,* Department of Biomedical Sciences, McQueary College of Health and Human Services
Rajiv Thakur, PhD: *Associate Professor,* Department of Geosciences, West Plains Campus

Faculty Reviewers

Dr. Deepti Agrawal
Dr. Holly Baggett
Dr. James Baumlin
Dr. Stephen Berkwitz
Dr. Bryan Brinkman
Mr. Marcus Cafagna
Mr. Joel Coltharp
Dr. Richard Garrad
Dr. Jamie Grigsby
Dr. Daniel Hellman
Dr. Lanya Lamouria
Dr. Etta Madden
Dr. Bukola Oyeniyi
Mr. Andrew Pilkenton
Dr. John Prescott
Dr. Kathy Pulley
Dr. Kyle Thomas
Dr. Alan Tinkler
Dr. Tonia Tinsley
Dr. Randi Ulbricht
Mr. Joseph Williams
Ms. Cynthia Winstead

Contents

Judith Enyeart Reynolds College of Arts and Letters

College of Humanities and Public Affairs

McQueary College of Health and Human Services

Judith Enyeart Reynolds College of Arts and Letters

"Dolly the Sheep and Mental Deception," "Freshmeat," & "Consumption"

Molly Del Rossi

Abstract

I wrote "Dolly the Sheep and Mental Deception" for my intermediate poetry course after a long drought of inspiration and a bout with depression. I looked around my dorm room, upset at my lack of motivation until I gazed upon my ratty sheep slippers with holes in the soles. This poem is focused on how the brain is a perfect saboteur. When I saw my twin slippers, I remembered learning about Dolly, the first mammal ever to be cloned from an adult sheep cell. She was living proof of the impossible, famous for her perfect replication; I found a strange connection to Dolly regarding our lack of self recognition. My use of dynamic voice throughout this poem is an attempt to envelop the reader through narrative and perspective. "Dolly the Sheep and Mental Deception" explores the stream of consciousness that is looking at your reflection and not knowing who is looking back at you.

"Freshmeat" began as scrawlings of text in the margins of textbooks during my freshman year at Missouri State. I was your typical homesick, scared, confused freshman who couldn't fathom surviving college away from the comforts I knew back home. I was in my Honors seminar when my professor jokingly asked if we think about how we might die, and that is the backbone of this poem. "Freshmeat" employs typical freshman life—dorm room heat, questionable dining hall food, and ghost stories—with a sinister undertone of rapid change feeling close to death. This poem was edited and finalized from crinkled notebook paper to my final poetry portfolio in December 2020. I used conventional left-hand margins, intruding on the white space in parentheticals that mirrored my secret doodles in those textbooks and scratch paper. I look back on this poem and am satisfied with how much my perspective has changed in comparison to the desolation of freshman year.

"Consumption" was started during the height of the Black Lives Matter movement in 2020 and finished in December of the same year; the issues of privilege and racism are timelessly pertinent, and a poem such as this one has been written a hundred different ways. I wrote this poem to analyze my point of privilege as a white woman who can only observe and sympathize with the struggle of racism in America. Examples of the narrator's experience of privilege are separated into four vignettes, taking common moments—family dinner, graduation, family heirlooms—and marking them with wickedness. The voice of this poem shifts multiple times from the perspective of an observer to a violent force that enacts their privi-

lege insensitivity, adamant denial of understanding, and theft of culture. The two voices are differentiated by italics. Though this poem is heavily metaphorical in imagery, the underlying message is the consistent theft from people of color by white people, who continue to turn their noses up and make a mockery of important racial movements. To be an ally, you must first recognize where you stand apart: your point of privilege, your history, and how you can cause change within your community. My attempt at this message in "Consumption" is framed by horror imagery and character voice.

Dolly the Sheep and Mental Deception

around this tiny house
i wear slippers.

two little sheep skins absorb the chill of the hardwood and the perspiration of my feet.
to keep the dust off their tufted white faces, i wash them weekly
and erase the pressure of my heels & arches,
the transfer of dirt that settled into my
soles.

taking out a translucent pink ruler, i compare last monday's imprint to this monday's imprint, my mirrored foot's depth is a fraction of a centimeter less than it was seven days ago, which means i'm walking lighter, thank the meals skipped, *adieu.*

two Dollys patter across the floor. these clean, cloned sheep lead my feet into daily erratic pacing, showering with my clothes on, dancing with the window open, fantasizing about doing hard drugs with these Dollys propped up on the dashboard & Vince Neil in the driver's seat.

i'm tip-toeing to the bathroom when i see their sewn faces
observe my absolute worst angle with an echoed smirk.

act of defiance: cut the ear off of one, stab a hole through its stitched eye, let the other watch.

i do her, my right slipper, a favor. *adieu*. i would be pissed too if someone cloned me by stowing away my DNA in little vials to make concrete the already present thought that Dolly wasn't enough.

yea, i'd maim that unnatural shadow of mine to show just how spiteful i am, ruin their little
science experiment, tell them to look my mom in the face and that should be proof enough
that god already replicates wills and passiveness and bumps in the bridge of the nose.

i give the false Dolly a viking funeral,

get its sherpa skin hot with a Vanilla Bean Noel scented wax block,
and float it in my bathtub with my hair clogging the drain in absence of a plug.

around this tiny house
i wear a slipper
and hear my bare left foot thump against hardwood.

Freshmeat

Diagnoses in bed late at night
when you have felt sick for weeks but the root cause was morphed
by months, days, miles and ticking seconds and flickering lights taped to
walls.

The humidity knocks them down every other night,
cinder block is unforgiving to pretty things.
Tiny stars reflect slow, twitching colors onto patchwork tile floors,
it's easiest to let them lie there until morning.

My professor asked us in pure perversion,
morbidity,
if we imagine our own deaths
every once in a while.

Timid hands shot up,
my blue nail beds looked translucent against bright fluorescents
and I looked around to wonder if they're sick
(too),
or if they see a blaze of fire against gravel roads,
or asphyxiation in the dining hall,
or an uncomfortable bed surrounded by people that tell you they love you
(you have to believe them at this point, anyway).

My fingernails say that I might disintegrate into the dirt,
let myself sink through the floor of seven stories

(the one beneath us is said to be
haunted
I have half a mind to believe
them)

(the other half went somewhere
without me)

and meet the worms
with my blue toenails
pointed up.

Consumption

I.

I saw my brother die once,
while playing a video game.
His beige walls echoed
with ruthless exclamations,
and he left the warfield
with the silent click of a button.

He went downstairs when my mother
chimed dinner.

A wide-eyed girl, opposite of my reflection,
saw her brother,
collapse massacred on the television
in high definition
and no mother was able to make
proper dinner
that night.

> *That windowpane brother that meets your eyes, but never hands.*
> *The kind that looks just like you when your face is full of fear.*

The meal served was a Dixie paper bowl,
full of tears and blood.
The little ones got their spoons
and closed the blinds.

II.

I was awarded a heart-shaped necklace
after graduation, from my father.

I saw another girl, streaming soot tears,
banner her daddy's teeth
around her neck.

> *What kind of jewels are those? I think they're beautiful, little girl, do you mind if I borrow them? Just for one night, one weekend maybe, if you would let me. I sure do think they're pretty and I just wish you would let me touch them, let me swish them in my mouth and I swear I'll spit them out when they're clean. I've never had a necklace like that.*

She cried when she adorned her
black gown.
She felt their beady red eyes,
like rats in a root cellar,
as they flashed their fleshy tails
and clapped their dirty paws.

III.

do you see people and pretend they are
shadows?

is it easy to squeeze out their breath when you
believe they will dissipate into light?

> *Are you sure I haven't seen you before? I swear I saw you face down on the television with a knee in your back. Wasn't you? Maybe your sister? Mother? Cousin? Niece? No? Damn, I could've sworn I saw your face when the light leaked from her tongue. It left a puddle on the ground and my buddy bottled it up to sell it at his store, you know the one. Some lady came in and bought it for a whole chunk of change, I'll tell you that. She hid it in her cheek when she left.*

IV.

My grandmother keeps a decorative, wooden box
with a mockingbird carved on the lid,
full of lungs and hearts and veins,
holes in backs and faces and children,

pull them out for everyone to see

look what you made me do this is what happens when you tell them our secrets you swore you wouldn't tell what we stole from them, now we have to carve their skin and wear it as a jacket.

The Princess Poems

Abigail Jensen

Abstract

"The Princess Poems" is a series of pieces investigating the profound anxieties of a young woman as she adjusts to adulthood and public participation. These poems explore this persona's journey through internalized misogyny, self-loathing, substance reliance, active imagination, and ultimately, hope from a surprising source. The style and voice of these pieces are influenced by the poetry of contemporary writers, such as Maggie Nelson, Kim Addionizo, and Jericho Brown, after whom "Seeing Red" is inspired. Nelson and Brown are challengers to the conventional structures of storytelling, and they both focus on social issues in a personal exploration. Addionizo draws much of her inspiration from her own experiences. All of my poems have a narrative element as the foundation for further examination of a young woman's existence and usefulness in her world. I usually start with a concrete image or scene when I begin to craft a poem, and later I come to find the speaker launching attacks on themselves and their compliance with inner hatred. Sometimes this battle ends brutally, with no conclusion, but occasionally some resolution, or at least acceptance, can be achieved in the end. In these poems, I have employed playfulness with lines, diction, and even a bit of prose to build a voice that seeks to resonate with an audience of adults in their 20s or any adults that have ever been 20. All the same, the search for one's highest "self" is too often obstructed by the culmination of many small insecurities, and these poems are obsessed with this thinking.

Princess

I've been tamed in all the ways I swore I never would be;
I placed one hand on my heart and the other on my mother's
moments before she unleashed my toddler legs and the
wild curls on our suburban lawn.

She locked me in a *Girl Power* tower and scraped out a canal
so I wouldn't wander into gentility; and yet, there came the first day
she demanded that I suspend apologies
for some offenses I never committed.

I was still sorry.

I just want to express empathy for the times young men approached me,
and I took them by the shoulders to turn them right back around.

The truth is that I fear them, but my mother can't know, she can't,
she just can't because she understands, as I do, that my cerebrum
is more mighty than all of theirs, but *Hello!*
I am just supposed to be utility here!

I offer my smile to Mr. Forty-Year-Old-Has-Been-Eyeing-Me-for-a-Block
because I can't say *I know!* when his *You're beautiful* drenches
my ears in saliva, or else what kind of pretty girl would I be?

I'm reminded of my dentist always calling me *Princess*
right before clamping my mouth open and digging around inside.

The keeper at the children's zoo calling me *Princess*
because I was eight and a little scared of the goats jumping my pelvis.

My high school drama teacher always calling me
Princess and referring to my high heels as *Fuck-me pumps*
but hey, I was almost sixteen.

I not-so-secretly hope there's another timeline where that girl from the lawn
listened to her mother, was not seduced by politeness, and kicked the shit
out of those third grade boys for saying *Your girl legs are too weak for kickball.*

But this is the dimension I'm stuck with,
the one where women can't be charming
and love themselves simultaneously; that's the conflict of the fourth wave.

We pretend we can row our little dinghies away from the beasts that continue to torment the waters through which we sail.

SHOT GLASS OF PANIC

i couldn't afford real glass
so i soaped and watered
my iron supplements out of their bottle,
and heart beatin whiskey
glug glugged its way on in.
these shake shakey hands of mine
seek to calm the flames in my brain
by starting a fire in my throat.
so i throw another one back.
i failed to replenish my
blood
with its metal oxygen, and so heat
cools my synapses with a snap!
i slip around on this too small couch,
and pour some more into the pill bottle of terror.
it's iron-deficient and full of whiskey,
like me.

Seeing Red

after Jerico Brown

I am sick of your sadness,
Miss Jensen, the clothes
scattered on your floor
and not in the sexy way.
I am tired of your exhaustion,
the refusal to take medication
that will boost the oxygen
in your already thin blood.
Do you think it will make
you all too energized?
Instead, you seek any excuse
to spend your last sweaty buck
on some overpriced shape of caffeine.
Your gorgeous hair is such
a menace to maintain, and it
slurps up all the products you own.
Seriously, frizz comes easy
whenever you yank curls
flat against your head so your
hair, or really, *you* are just a bit
smaller. And then there's the little
time you allow yourself to eat,
your empty stomach,
your obsession with your ass;
what the hell is that about?
Stop telling your roommate the details.
I'm over it with the
way you bully yourself.
Every other sentence is self-deprecating,
and you can't even
pretend that the cruel thoughts you have
are spoken by an undisclosed "voice."
It's just you, you whore.
I know you've got a brain
that must have been
shattered

and super glued back together,
but that's no reason to mope
more than any other twenty
something; do you ever consider
just cleaning your room, getting laid,
pouring all these feelings in a flask
like everyone else? That may be what
the experts advise against,
but hey, Miss Jensen—
can I call you
Abby?—it might just
be what you can do for
now.

A Burning Question

Why is there so much
sickness in your sphere?

You, poor child, fail to freeze
those tears upon your face

so your bottom lip protrudes
and shudders without control.

The hollow pits of your stomach
cry too while gripping your throat.

And as your fingers jab inside
your very own mouth,

pained gags release the distress
from within you, plus some blood.

There is no comfort in your sheets
at three in the morning,

so your car must speed down
the ghostly street to the gas station.

You wrap your fingers around
a Canada Dry, or two for the price of one.

You may feel its sweetness automatically
pump more skin upon your cheeks

the moment you allow the bubbles
to wiggle down your pipe.

Upon return to your loose-leaf-cluttered
bedroom floor, you turn away from the mirror

and guard your guise against the beige
notebook resting on the carpet next to an RBG figurine.

Little Ruth fell from the bookshelf, and your limp
wrists can't bear to hoist her on her pedestal again.

After all of this, you refuse
to mark your thoughts in blue ink.

Why panic at the sight of
the oddity that you are?

Why not enjoy the chaos
of your own nerve endings?

Surrounded by Clones in a Dream

The premise of my life is a kind of Scottt Pilgrim scenario.
Instead of destroying seven deadly exes or whatever,
it was up to me to locate and subdue some imposters.
The first, an office administrator at my
college who said, "Okay, work! Yes,
momma!" when I tried to schedule an
advising appointment.
I can't lie. She was hard to take down, but I'm scrappy.
WHACK! a PVC pipe against Kathleen's noggin.
Can't say where the pipe came from. The continuity in this story sucks.
Then, as dreams go, I was *PLOP!* at a random party,
needing to piss, and a fraudulent Molly-from-history-class lay
naked on the bathroom floor with a mysterious
shaved head between her legs. Never saw his face though.
I defeated clone Molly by pinky-promising I wouldn't tell a soul.
BLINK! I materialized in the hallway of a school building
where my boyfriend was dressed like someone who had
tried to dress the way he does, but they couldn't find the right
kind of forest green t-shirts and corduroy pants and instead gave up.
The other clue was that clone Cole couldn't seem
to figure out who the fuck I was so I lured his ass
to a Target and *CLICK!* locked him in a storage space.
Through all these adventures, I never got to meet a clone
of Me. I would have befriended her and trained her to become
the Me that everyone else sees. She'd be the one
to *BEAT!* that potato face with contour each morning
and taste those gluey, teeth-whitening strips at night.
She'd be the voice that tells my parents over the phone
that my car's been towed and two weeks pay has gone to waste.
She'd get to say, "I don't give a shit" when I have to miss
Thanksgiving and *REHEAT!* chicken nuggets instead.
She'd feel what all this does to *SLAM!* her pigeoned-chest body.
But I suppose Clone Me already does.

The Invisible Romantic: The Life and Legacy of Fanny Mendelssohn-Hensel

Kyle Evan Hulsebus

Abstract

This research paper depicts the life and legacy of Fanny Mendelssohn-Hensel in correlation with her most famous musical works. Fanny Mendelssohn was the older, lesser-known sister of Felix Mendelssohn, the prolific Romantic era composer of German lied. Her contribution to the development of German music and involvement in Felix's compositional career easily marks her as the most important female composer working in Germany during the 1800s. This paper reviews major life events, starting with her enrollment in the Berlin Sing-Akademie music society in 1820 and concluding with her untimely death during a rehearsal of Felix Mendelssohn's work in 1847. Each major life event is associated with several songs Fanny composed at the time, which are dissected thoroughly in this research paper. The primary goal of this project is to raise awareness of Fanny Mendelssohn's extraordinary life and contribution to German lied.

Introduction

The eldest child of Abraham and Lea Mendelssohn, Fanny Mendelssohn was born in Hamburg, Germany in 1805. A direct descendant of the renowned philosopher Moses Mendelssohn, Fanny came from a long line of influential and distinguished Jewish figures. Her fascination with musical performance was established at a very young age, and she began studying keyboard with her mother before the age of twelve. At age fourteen, Fanny began composing music, though she was untrained at the time. Although many of her early works have been lost to time, Fanny's first dive into composition depicted an emulation of Bach's compositional approach to keyboard performance. Even before enrolling in music school, her work displayed a sense of curiosity associated with chromaticism and hypermeter (Christian).

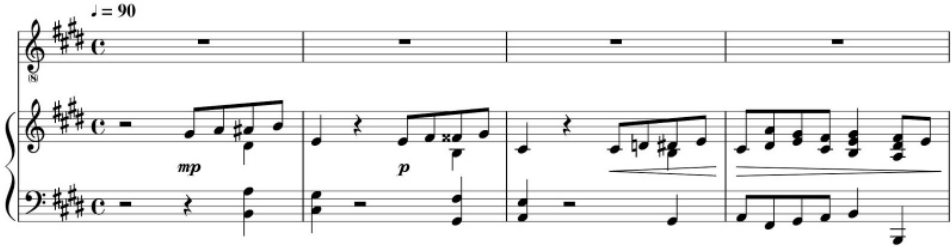
Early Life in Berlin

Education - Erster Verlust I

Fanny and Felix joined the Sing-Akademie music society in Berlin in 1820, which designates *Erster Verlust I* as the beginning of Fanny's academic training in music composition. Although supportive of her passion, Fanny's father wrote to her in 1820 saying, "Music will perhaps become his [Felix's] profession, whilst for you it can and must be only an ornament." As a young woman living in the early 1800s, Fanny was subject to the gender norms and societal expectations of nineteenth-century Germany, which preventer her from fully pursuing her musical talents. Nonetheless, Fanny and Felix began studying piano under Ludwig Berger and composition under Carl Freidrich Zelter; both mentors communicated their admiration for J.S. Bach's work to the siblings throughout their academic studies. This led to Fanny's later fascination with Bach, going as far as memorizing all twenty-four preludes from his Well-Tempered Clavier within her first couple of years at the Sing-Akademie (Christian).

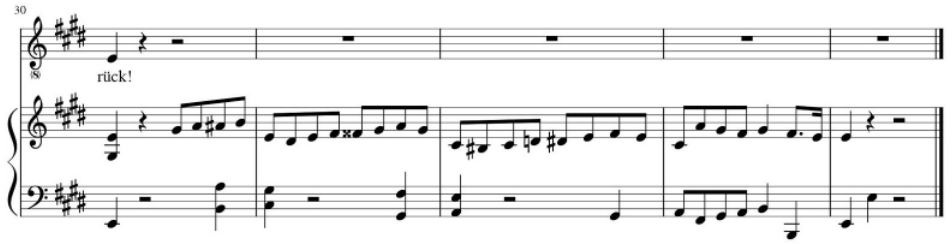
Much of Fanny's earlier work takes after Bach's preludes in both lyricism and chromaticism, and her piece *Erster Verlust I* is no exception. The contrary motion between vocal melody and the right hand of the keyboard establishes unique harmonic expressions. Fanny's first major composition accurately displays her prowess with the keyboard, including the presentation of a counter melody before the singer even enters. An interesting section of this work is the subversion of expectation in the keyboard line within the last five measures. She set up a specific recurring melodic figure in mm. 1–5 …

Erster Verlust I, mm. 1–5 by Fanny Mendelssohn-Hensel (1805–1847)



… only to slightly change this figure by adding melodic content between the motives at the end of the composition to vary the repetitive nature of the A sections, as well as bring the entire piece to a satisfying conclusion:

Erster Verlust I, mm. 30–34 by Fanny Mendelssohn-Hensel (1805–1847)



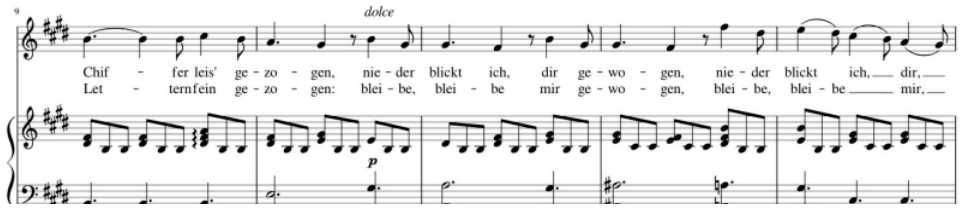
Misattribution - Suleika und Hatem

Over the next five years, it became obvious to the Mendelssohn family that Fanny's keyboard and compositional skills matched those of her brother. In a letter introducing the poet Johann Wolfgang von Goethe to Abraham Mendelssohn, Carl Freidrich Zelter wrote Abraham "has adorable children and his oldest daughter could give you something of Sebastian Bach," and "this child is really something special" (Conway 171). Felix was not outwardly supportive of his sister's efforts due to their parents' disapproval, but he would often consult Fanny with questions regarding his own compositions ("Fanny Mendelssohn"). A letter from Fanny regarding her younger brother in 1822 read, "I have always been his only musical advisor, and he never writes down a thought before submitting it to my judgment" (Hensel, vol. 1 117). By the mid-1820s Fanny and Felix were working together to publish some of her work under his name. In particular, several pieces appearing in Felix's Op. 8 and 9 were in fact written by his sister (Todd, A Life in Music 174), including *Sehnsucht, Verlust, Suleika und Hatem, Italien,* (Todd, *A Life in Music*, 175, 86, 252) and several others. Of these compositions, *Italien* became the most popular of Felix's Op. 8, which led to an embarrassing interaction in

1830 when Felix had to explain to Queen Victoria that her favorite Mendelssohn composition was actually written by his sister (Hensel, vol. 2 168–171). Needless to say, many of Fanny's songs received high praise throughout the 1820s, though her name was not attached to them.

Suleika und Hatem holds the title of Fanny's first vocal duet composition and was only her second time writing for the male voice. This piece signals a major turning point in Fanny's compositional career as she begins to utilize harmony as opposed to rhythm to destabilize the B section of her works. Vast lyricism and chromaticism return in this song with an added focus on rhythmic stability, visible in mm. 9–13 as the keyboard line as she drastically pars down the keyboard line to provide a steady pulse for the soprano melody to soar above:

Suleika und Hatem, mm. 9–13 by Fanny Mendelssohn-Hensel (1805–1847)



This effect is also achieved via the introduction of choral doubling—a feature in which the keyboard imitates the vocal line in conjunction with the singer—to serve as a unifying tool in the duet, but also an aide in pitch placement for the singers. Suleika und Hatem was one of the first appearances of said technique in Fanny's vast catalogue of lieder.

Fanny's first take on the duet genre is quite different from the typical classical duet. Whereas the soprano presents the plot of the story, the tenor merely interjects his emotions regarding the events at the end of the piece. The audience is introduced to two unique perspectives of this relationship as well as a brief intersection of the two perspectives in one grand polyphonic gesture.

Concerts and Letters - Wiederfinden

At an early age, Fanny's younger brother Felix began garnering critical acclaim for his compositional technique, leading to vast performance and travel opportunities for the young virtuoso. During this time, the two Mendelssohn siblings maintained steady correspondence, resulting in hundreds of letters between the two. Felix, who still considered Fanny his foremost compositional advisor, was never shy in delivering new works to his older sister for critique ("Fanny Mendelssohn Hensel"). In his time abroad, Felix frequently found himself accompanying vocalists performing his original works, and it

was at this time that Fanny began seeing the success of her early compositions published under her brother's name (Christian).

In the early 1820s, Fanny's musical priorities began to shift as her lieder started directly reflecting her life experiences. She soon became seemingly more stringent with her process of deciding which texts to accompany her work as she sought to share her biographical life through her compositions. Since she only started keeping a personal diary in 1829, many of her thoughts and feelings during the formative years of her life are a mystery (Christian). What the public does have access to is her vast catalogue of lieder, which began growing in the 1820s as she moved away from keyboard literature in an effort to immerse herself in the genre of German lied, a compositional method that sets poetry to classical music. Felix's fame became a useful tool for Fanny to publish her works and therefore share her story with the world.

Wiederfinden was composed in 1825 and began Fanny's biographical musical employment. Set to text by Johann Wolfgang von Goethe (Paley), this composition explores separation, the desire for intimacy, and the depression associated with both feelings. The text paints the image of an individual pining for the return of a loved one. Upon their partner's return, the narrator rejoices in the unfailing eternal bond of two lovers while the narration warns about what may come when separation occurs again:

Ist es möglich! Stern der Sterne,	It is possible, star of stars,
Drück ich wieder dich ans Herz!	That once more, I press you to my heart!
Ach, was ist die Nacht der Ferne,	Oh! What pain has our night apart
Für ein Abgrund, für ein Schmerz!	Brought in its abyss!
Ja, du bist es, meiner Freuden	Yes! It is you and of my joys
Süßer, lieber Widerpart!	The sweet and dearest counterpart;
Eingedenk vergangner Leiden	But mindful of those sorrows past,
Schaudr ich vor der Gegenwart.	I tremble at the present.

Translated by John Storer Cobb (1838–1904)

Although on the surface this work can be viewed as a plea for romantic affection from Fanny, it makes more sense to read the eternal bond the text describes with the Mendelssohn siblings' relationship. Fanny and Felix were deeply connected by both their passion for music and their shared life experiences in their younger years. The hundreds of letters exchanged between the two siblings during Felix's travels point to Fanny's struggles with separation from her brother, which led to those emotions being more prominently displayed in her biographical lieder ("Ist Es Möglich! Stern Der Sterne").

Midlife, Marriage, and Travel

Love and Wonderlust - Italien, Über allen Gipfeln ist Ruh

In 1821, Fanny Mendelssohn was sixteen when she met her future fiancé, Wilhem Hensel. A painter at the time, Hensel traveled across Europe to create original portraits of royal figures. In 1822, Fanny began composing music set to Hansel's poems (Christian). In addition to his status as a painter, Hensel was also known for his interest in poetry, at one point considering abandoning painting altogether to pursue literary arts ("Wilhelm Hensel"). Although their love for one another was public knowledge, the Mendelssohn family feared their reputation in the Lutheran church would be tarnished by allowing Fanny to be courted by a Catholic man (Buja). Thus the many compositions set to Hensel's poems were hidden from the rest of her family and only debuted after she got married.

In 1825, King George III requested some copies of Raphael's most famous works for his own collection, sending Hensel to Italy to complete the project ("Wilhelm Hensel"). Fanny and Hensel maintained letter correspondence over the next three years, and it was through these letters that Fanny developed an admiration for and longing to visit the Italian countryside. Her most famous work, *Italien*, reflects her intense desire for travel and adventure. Whereas her brother and future fiancé had the opportunity to travel across Europe pursuing their respective careers, Fanny's parents discouraged her from doing the same (Christian).

The text of *Italien* was written by Franz Grillparzer ("Fairer and Fairer"), a prolific librettist of the Romantic era working during the mid-1800s. The lyrics and translation of Italien's text are as follows:

Schöner und schöner Schmückt sich der Plan, Schmeichelnde Lüfte Wehen mich an!	Fairer and fairer the plain becomes, As caressing breezes blow on me!
Fort aus der Prosa Lasten und Müh' Zieh ich zum Lande Der Poesie.	Away from the burden and trouble of prose, I go forth into the land of Poetry.
Goldner die Sonne, Blauer die Luft Grüner die Grüne, Würz'ger der Duft!	More golden the sun, more blue the air, More green the green, more aromatic the scents!
Dort an dem Maishalm, Schwellend von Saft, Sträubt sich der Aloe Störrische Kraft!	There on the corn grass, swelling with sap, The aloe bristles with stubborn strength!

Ölbaum, Cypresse, Blond du, du braun,	Olive, Cypress, one light and one dark,
Nickt ihr wie zierliche Grüßende Fraun?	Are you nodding like dainty, greeting women?
Was glänzt im Laube, Funkelnd wie Gold?	What is gleaming in the leaves, glittering like gold?
Ha! Pomeranze, Birgst du dich hold?	Ha! Oranges, are you lovely ones hiding there?

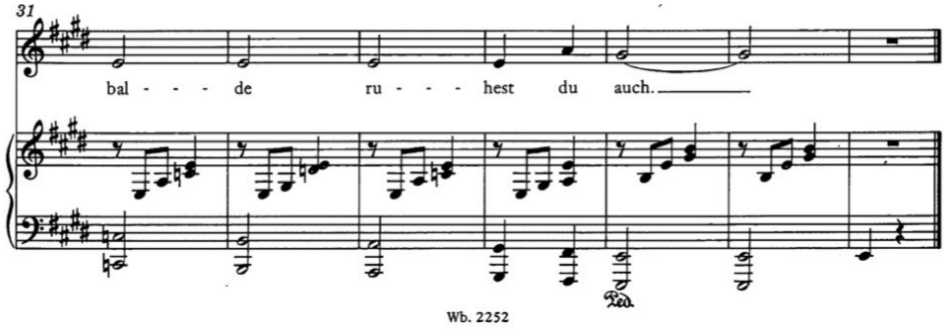
Translated by Emily Ezust (b. 1972)

Fanny may have chosen this poem for her first composition tackling her immense wanderlust because Grillparzer depicts a desire for greener pastures from the perspective of a lyricist. Fanny was likely drawn to the text because she could personally relate to the desire to be and experience more in life. With almost her entire family voicing their dissent toward her passion for music, it follows that she would feel confined and obstructed from reaching her goals. The imagery of Italy constructed through letters from Wilhelm depicted a vast countryside where she could finally find freedom from the subjugation and oppression of her gender during the nineteenth century. Fanny likely saw Italy as an escape from her current life and set her aspirations accordingly.

Fanny composed *Italien* in 1825 while on a family trip to Switzerland, and it was not until this trip that she finally satisfied a portion of her wanderlust. Both Fanny and Felix attempted to convince their parents to travel to Italy on their family trip to no avail . On the return trip from Switzerland, the family stopped in Weimar, Germany to visit with Johann Wolfgang von Goethe. A close friend to Abraham and Lea, the famous texts of Goethe were set to many songs by the Mendelssohn siblings. Their admiration and respect for Goethe's literary and theatrical work rivaled that of Bach. It was during this visit that Fanny performed several of her own settings of Goethe's work in his own home (Christian), winning his favor and prompting the response "she plays like a man" (Todd, *The Other Mendelssohn*, 146). This was high praise for a female musician in the 1800s, and Fanny took his words to heart. Goethe would go on to dedicate several poems in Fanny's honor, which she in turn set to music.

Of the many songs Fanny Mendelssohn set to Goethe's poems, *Über allen Gipfeln ist Ruh* stands above the rest. Written in 1780 and officially published in 1815, the text of this poem garnered its fame from Franz Schubert's setting in 1822 under the original title *Wandrers Nachtlied II* ("Wanderer's Night-song"). Although satisfying many of her desires for travel, the Mendelssohn family trip to Switzerland also invigorated her intense wanderlust. She depicts her desire to explore the world in her composition of *Über allen Gipfeln ist Ruh* alongside a resolution of peace presented in the last line of the song:

Über allen Gipfeln ist Ruh, mm. 31–37 by Fanny Mendelssohn-Hensel (1805–1847)



A translation of the last line in *Über allen Gipfeln ist Ruh* by Richard Stokes reads "The little birds are hushed in the wood. Wait, soon you too will be at peace" (Goethe,"Wandrers Nachtlied II"). This line serves as an excellent example of Fanny's life view from this point forward. Having experienced what may be the closest thing she would get to a sojourn in the Italian countryside, Fanny resigned herself to building a life with Wilhelm Hensel.

Wed to Wilhelm - Der Strauß

Fanny's first diary entry was written on January 4, 1829. Her brother, Felix, had found great success in his revival of Johann Sebastian Bach's *St. Matthew Passion* and thus began spending less and less time at home. It became evident to Fanny that she and her brother were slowly growing apart, prompting her first diary entry to read, "Felix, our soul, is leaving; the second half of my life stands before me." Nineteen days later, Hensel returned from his five-year trip to Italy and promptly proposed to Fanny after his appointment as royal court painter of Prussia. The two began planning their wedding, which would take place on October 3, 1829 (Christian).

Fanny had requested her brother write a processional for her wedding day; a piece that unfortunately would not come to fruition due to a leg injury Felix incurred soon after. Fanny took it upon herself to compose a processional and recessional for her own wedding only a few days in advance, now considered her first surviving compositions for the organ. The wedding was in the Parochialkirche, a reformed church in Berlin, where friends and family gathered to celebrate the spiritual union of Fanny and Wilhelm. Felix, however, was absent, a point that pained Fanny for years (Christian).

A month after the wedding, Fanny became pregnant with their first and only child, Sebastian Ludwig Felix Hensel. Sebastian was born one month

premature on June 17, 1830, and took up much of Fanny's time over the next couple of years. In fact, 1831 was Fanny's least productive year for composition, only producing seven new works after recovering from Sebastian's birth. Regardless, Fanny and Wilhelm's child brought them immense joy throughout the coming years, and they grew closer through raising Sebastian. She described their marriage in great detail. Her diary once read: "It seems impossible to us that love and happiness could grow even greater, but so it is, that with every day we become closer, and every night we agree how happy we are." It was this euphoria that quickly launched Fanny back into her work, where she began shifting from piano to voice as her primary instrument of choice in 1832 (Christian).

Inspired by her brother's success with different compositional genres and influenced by her husband's constant support, Fanny began expanding her range of musical works in the mid-1830s. Delving into concert aria, orchestra, and chamber chorus, Fanny's repertoire diversified over the next decade. After years of wondering if her work meant anything, she experienced newfound encouragement at the publication of her song *Ave Maria* in 1832 under her own name (Christian). The year 1835 saw many duets composed by Fanny, several of which lacked keyboard accompaniment. One such song, *Der Strauß*, featured the return of Fanny's biographical composition style in a new genre. Set to text by Johann Wolfgang von Goethe (Goethe, "Blumengruß"), *Der Strauß* serves as a greeting between loved ones and was likely composed in honor of Hensel. His support of Fanny's passions over the span of her career encouraged her to continue working, even through the early years of Sebastian's life. The song serves as a testament to her love and appreciation for her husband, the man who helped her overcome so many barriers of the time period (Christian). The text is as follows:

Der Strauß, den ich gepflücket	May this garland I have gathered
Grüße dich viel tausendmal!	Greet you many thousand times!
Ich habe mich oft gebücket,	I have often stooped down,
Ach, wohl eintausendmal,	Ah, at least a thousand times,
Und ihn ans Herz gedrücket	And pressed it to my heart
Wie hunderttausendmal!	Something like a hundred thousand!

Translated by Emily Ezust (b. 1972)

Notice just how simple the text of this poem is. It follows an ABAB rhyming scheme, one of the most elementary approaches to poetry. Despite its simplicity, this short composition is remarkably through-composed—varying its melodic material from sentence to sentence. This type of compositional approach, repopularized by Franz Schubert in the early 1800s ("Franz Shu-

bert"), allowed Fanny to create an interesting, continually unfolding melody to partner Goethe's simple text.

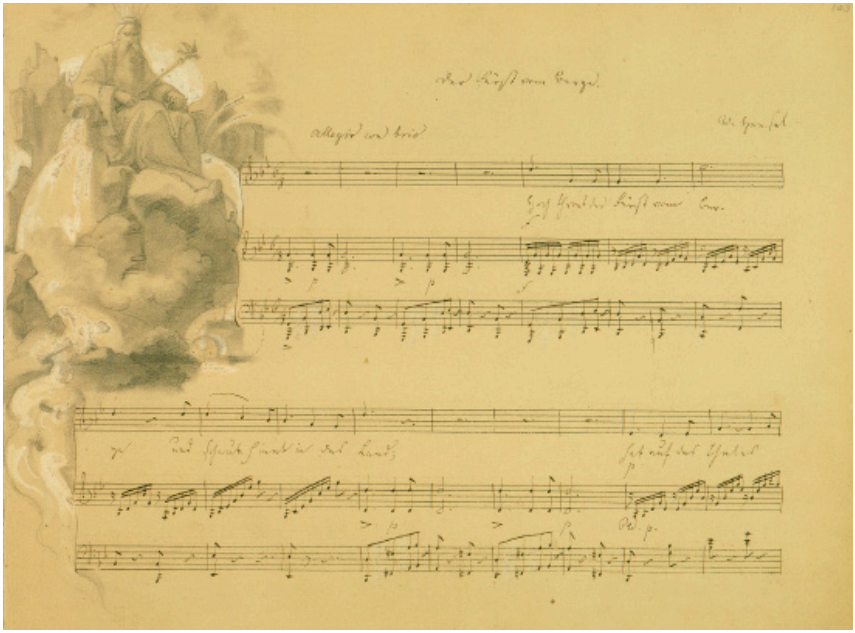
Dreams Come True - Der Fürst vom Berge

In August 1839, Fanny was granted her lifelong wish when Hensel decided to take the family on a year-long trip to Italy ("Fanny Hensel Born Mendelssohn").The couple and their young son began the strenuous journey from Berlin to the Italian countryside, stopping only briefly in Leipzig to stay with Felix. It was during this trip that Fanny experienced some of her first professional recognition and became acquainted with many talented musicians working in Italy at the time (Christian). One such musician was the influential French composer Charles Gounod, who once remarked, "Mrs. Hensel was an extremely learned musician and played the piano very well. Despite her small, slight figure she was a woman of excellent intellect and full of energy that could be read in her deep, fiery eyes. Along with all this, she was an extremely talented pianist" ("Fanny Hensel Born Mendelssohn"). For the first time in her nearly thirty-year career, Fanny finally felt as though her creativity paid off.

Fanny never ceased composing during the family's residency in Italy. In fact, 1839–1840 saw the most dramatic increase in compositions since her early years at the Sing-Akademie. Throughout their stay in Italy, Fanny and Wilhelm worked together to create a scrapbook of their travel experiences that would later be bound together with a title page reading, "Travel Album: 1839–1840." This cooperative work included German lieder, piano solos, poems, and illustrations and remains the only surviving display of their combined talents (Buja). Of the eighteen compositions depicted in this album, only five are written for voice and piano. *Der Fürst vom Berge* is a recently uncovered work presented in the Hensel travel album and may be the only surviving copy.

Der Fürst vom Berge, or "The Lord of the Mountains," was likely written during the family's return trip through the Alps. The song depicts a corrupt ruler who makes decisions on behalf of his people, in spite of their disapproval. The text serves as a nod to the polarizing king of Prussia, Friedrich Wilhelm IV, who at the time enacted policies frowned upon by the general public (Stokes). The nursery rhyme-stylized verses, coupled with the martial rhythms of the main theme, set high expectations for the character of this piece, which are quickly eradicated by the eerie harmonic gestures and unstable hypermeter present throughout the song. The listener is left with a bitter taste in their mouth as the piano motif slowly fades to nothingness at the conclusion of this work. On the next page is a copy of the title page for

Fanny's *Der Fürst vom Berge* with a pencil-sketch vignette by Wilhelm.



Hensel, Fanny and Wilhelm Hensel, *Der Fürst vom Berge* in *Travel Album*, 1839–40, facsimile on canvas. bpk Bildagentur, Mendelssohn Archive, Berlin State Library, Prussian Cultural Heritage Foundation, Berlin, Germany. Art Resource, NY.

Late Life and Legacy

Publication - Schweigend sinkt die Nacht hernieder

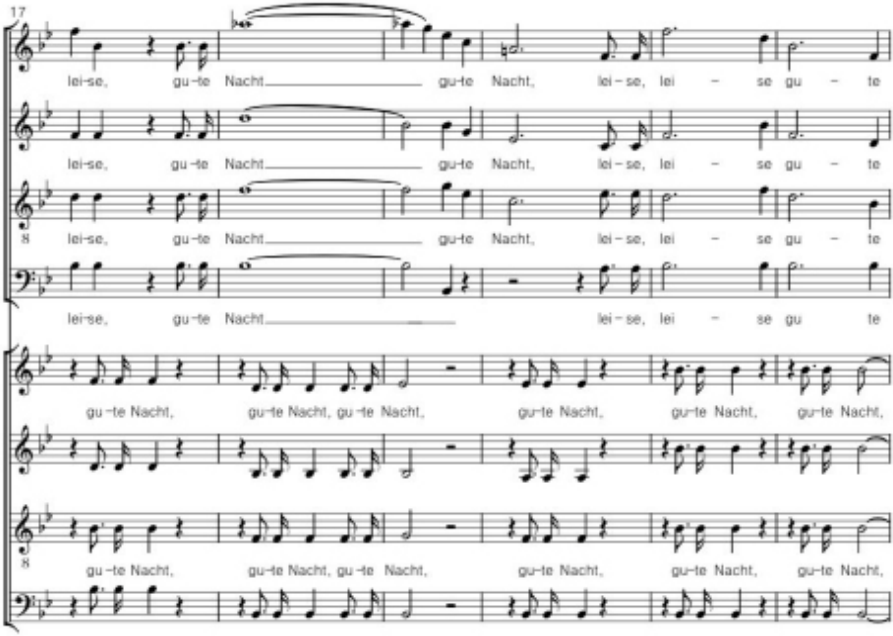
Fanny added eleven new vocal quartet compositions to her repertoire in 1846. With the creation of a small vocal ensemble that met at the Hensel household every week, Fanny began debuting her quartets to the world. It was during this time that she met the German diplomat and accomplished pianist Robert von Keudell, who grew fond of her work and served as her informal advisor and editor. Von Keudell desired to share Fanny's compositions with the world, and it was his and Wilhelm's encouragement that led to Fanny's decision to publish her work. Countless letters were sent to Felix during this time, but many were left unanswered as he was engaged in the process of translating his oratorio, Elijah. Felix had expressed his concerns earlier in life with Fanny's publication endeavors, citing their parents' disapproval (Christian). Regardless, Fanny pushed onward. She had finally learned to ignore the oppressive voices in her life in pursuit of her musical aspirations.

Fanny sought to distinguish herself from her brother as much as possi-

ble, and therefore refrained from publishing with Breitkopf und Härtel—the company Felix had worked with for many years. She instead turned to von Keudell as a private publisher and worked closely to begin cataloging her early work for editing. Getting to share her music, Fanny finally felt validated and rejuvenated as a composer. She began work on new compositions and eventually produced fifty new works in the span of just a few months. Despite this influx of compositions, Fanny still decided to publish her early work first, saving her newest creations for later opuses. She never received Felix's full approval in this endeavor, but one letter exchanged between the two showed him congratulating her hard work and success (Christian).

Of the new works she put forth in 1846, it was Fanny's quartets and octets that defined the revival of her musical curiosity, and *Schweigend sinkt die Nacht hernieder* is no different. She begins the piece with chorus one setting up the main theme before introducing chorus two to vary the theme. Chorus two utilizes fragmented material from the main theme to fill in empty spots in chorus one's melody while simultaneously overlapping with chorus one to provide interesting contrast between the two parts. This can be seen in the excerpt below:

Schweigend sinkt die Nacht hernieder, mm. 17–22 by Fanny Mendelssohn-Hensel (1805–1847)



This overlapping of material is slowly and meticulously entwined as to join the two voice parts at the end of the piece, and it is not until that point that

chorus two finally gets to share the full melodic line with chorus one. Both choruses are granted equal value in this composition as each strives to accomplish a different goal in the soundscape. Chorus one presents new thematic ideas as chorus two evaluates and modifies the ideas to keep the piece moving forward.

Untimely passing - Bergeslust

Consumed with the intricacies of daily life and family, Fanny slowly curtailed the output of new compositions in early 1847. Her final diary entry read, "I fear that I stand at the end of my publishing business," but that point was neither labored upon nor further clarified. Frequent visits from the Schumann family led to an increase in performances of Fanny's music at the Hensel residency, including some of her recently published trios. Additionally, Fanny began revisiting her brother's old symphonies for use in the family's Sunday music concerts. On May 14, 1847, Fanny was rehearsing her brother's *Die erste Walpurgisnacht* for performance the following day when she experienced a series of strokes while at the keyboard. She passed away that night in her home with her family and friends present (Christian).

When Felix received word of his sister's passing, he broke down. On his last trip to Berlin, he visited the Hensel residence to pay his respects to the most respected music advisor he ever had. It is no coincidence that Fanny's final compositions appeared under the Breitkopf und Härtel publishing label as he spent the last five months of his life dedicated to publishing the last of her compositions. He may not have outwardly recognized or praised Fanny's compositional skills in life, but Felix's actions depict his admiration and respect for his late sister's work. Felix passed away on November 4, 1847 from the same cause as his sister, but the grief associated with the loss of his sister killed his passion for composing much sooner (Christian).

Of Fanny's final compositions published by her brother, *Bergeslust* was completed the night before his untimely passing. The song, set to text by Joseph von Eichendorff, is an upbeat and joyous recollection of travel (Eichendorff), serving as a fitting end for Fanny's experiences with wanderlust and her sojourn in Italy. Although reminiscent of her early music and compositional style, the piece provides a flawless bookend to Fanny Mendelssohn-Hensel's remarkable life. When listening to her final composition, one can hold fast to the concluding line of text and consider how her story will continue to live on through her music:

"Thoughts and songs go winging on till they reach the kingdom of heaven."

Works Cited

Buja, Maureen. "Musicians and Artists: Mendelssohn and Wilhelm Hensel." *Interlude*, 21 Apr. 2019, https://interlude.hk/musicians-artists-mendelssohn-wilhelm-hensel/#.

Christian, Angela Mace. "Hensel [Née Mendelssohn (-Bartholdy)], Fanny Cäcilie." *Grove Music Online*, Oxford UP, 28 Nov. 2018, https://doi.org/10.1093/omo/9781561592630.013.3000000159.

Conway, David. *Jewry in Music: Entry to the Profession From the Enlightenment to Richard Wagner*. Cambridge UP, 2012. *EBSCOhost eBook Collection*, search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=nlebk&AN=416708&site=eds-live&scope=site.

Eichendorff, Joseph von. "Bergeslust: Song Texts, Lyrics & Translations." Translated by Richard Stokes, *Oxford Lieder*, Oxford Lieder Limited, 2005, www.oxfordlieder.co.uk/song/5170.

"Fairer and Fairer (Grillparzer, set by (Felix Mendelssohn (Misattributed), Fanny Mendelssohn-Hensel))." Translated by Emily Ezust, *The LiederNet Archive: Texts and Translations to Lieder, Mélodies, Canzoni, and Other Classical Vocal Music*, The LiederNet Archive, Sept. 2003, www.lieder.net/lieder/get_text.html?TextId=6893.

"Fanny Hensel Born Mendelssohn." *Fanny Hensel Biografie*, Furore Verlag, www.fannyhensel.de/hensel_eng/bio_frame.html.

"Fanny Mendelssohn." *Wikipedia*, Wikimedia Foundation, 8 Feb. 2021, https://en.wikipedia.org/wiki/Fanny_Mendelssohn.

"Fanny Mendelssohn Hensel, 1805–1847." The Library of Congress, www.loc.gov/item/ihas.200156440/.

"Franz Schubert." *Wikipedia*, Wikimedia Foundation, 5 Mar. 2021, en.wikipedia.org/wiki/Franz_Schubert.

Goethe, Johann Wolfgang von. "Blumengruß: Song Texts, Lyrics & Trans-

lations." Translated by Richard Stokes, *Oxford Lieder*, Oxford Lieder Limited, 2005, www.oxfordlieder.co.uk/song/2498.

Goethe, Johann Wolfgang von. "Wandrers Nachtlied II: Song Texts, Lyrics & Translations." Translated by Richard Stokes, *Oxford Lieder*, Oxford Lieder Limited, 2005, www.oxfordlieder.co.uk/song/713.

Hensel, Fanny and Wilhelm Hensel, Der Fürst vom Berge. 1840, facsimile on canvas. OMI: Old Manuscripts & Incunabula, 2021. https://www.omifacsimiles.com/brochures/hensel_furst.html.

Hensel, Sebastian. *The Mendelssohn Family: 1729–1847*, vol. 1, Greenwood Press, 1968.

Hensel, Sebastian. *The Mendelssohn Family: 1729–1847*, vol. 2, Greenwood Press, 1968.

"Ist Es Möglich! Stern Der Sterne (Goethe, set by (Fanny Mendelssohn-Hensel, Karl Friedrich Zelter))." *The LiederNet Archive: Texts and Translations to Lieder, Mélodies, Canzoni, and Other Classical Vocal Music*, The LiederNet Archive, www.lieder.net/lieder/get_text.html?TextId=45718.

Paley, David. "Poems in Translation: Wiederfinden." Translated by John Storer Cobb, Poems Without Frontiers, www.poemswithoutfrontiers.com/Wiederfinden.html.

R. Larry Todd. *Fanny Hensel: The Other Mendelssohn*, Oxford UP, 2010.

R. Larry Todd. Mendelssohn: *A Life in Music*. Oxford UP, 2003. *EbscoHOST eBook Collection*, https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=nlebk&AN=120958&site=eds-live&scope=site&custid=039-820.

Stokes, Laura. "Music Talk with Laura Stokes: 'Fanny Hensel's Lied "Der Fürst Vom Berge" in Light of Contemporary European Politics.'" *Brown University Department of Music*, Brown University, 2 Nov. 2020, www.brown.edu/academics/music/events/music-now-talk-laura-stokes-

%E2%80%9Cfanny-hensel%E2%80%99s-lied-%E2%80%98der-f%C3%BCrst-vom-berge%E2%80%99-light-contemporary-eur.

"Wanderer's Nightsong." *Wikipedia*, Wikimedia Foundation, 24 Jan 2021, https://en.wikipedia.org/wiki/Wanderer%27s_Nightsong.

"Wilhelm Hensel." *Wikipedia*, Wikimedia Foundation, 12 May 2020, en.wikipedia.org/wiki/Wilhelm_Hensel.

Power in Portraiture: An Examination of Isabella d'Este's Self-Portrayal Through Art

Whitney Mosley

Abstract

Through her calculated self-fashioning of images that portrayed her as well-educated, cultured, and powerful, Isabella d'Este used portraiture to gain access to the typically male-exclusive spheres of the fifteenth-century Northern Italian court. Through an iconographic examination, this paper inspects the portrait drawing of Isabella done by Leonardo da Vinci, comparing this image with those of her created by Gian Cristoforo Romano, Francesco Francia, and Titan. While previous scholarship surrounding Isabella d'Este's art patronage discusses her use of portraiture to uniquely elevate her political and social status, this research works to uncover her intentional display of specifically masculine elements of portraiture, combined with representations of her feminine beauty, to assert her authority as a female ruler. This examination of her involvement in the development of these portraits, building on the scholarship surrounding her correspondence with court artists, reveals Isabella's own awareness of the important effect these representations had on her status at court. A discussion of her dissemination of these images as gifts to members of her own court at Mantua and other Italian courts proves her use of these portraits to propagate an image of herself that enabled her to achieve greater power and influence. This paper reveals the ways in which Isabella used Italian Renaissance portraiture to intentionally fashion images of herself that both flattered her physical appearance as a woman and represented qualities of her personal character and status as a ruler, portraying her as a virtuous, culturally superior, and powerful leader.

Introduction

Isabella d'Este was expected to perform a supportive role to her husband. Born to the ruling family of Ferrara, Italy, and married to Francesco Gonzaga, the ruling nobleman of the northern Italian city of Mantua, Isabella's primary duties included providing her husband with a male heir and supporting an alliance between the Estes and the Gonzagas. Over the course of her life from 1474 to 1539, she was not only successful in these endeavors, but she surpassed expectations for an elite woman of the fifteenth century, fostering social and political power independent of her husband. Today many scholars interpret her role at court as an acting co-regent of Mantua, a ruler equal to her husband in status and power.[1] Isabella was also a prolific art collector, and her patronage of the arts allowed her to enter social and political spheres typically occupied solely by men of the court. Never passive in her role as political and cultural leader, she was known for providing artists with feedback and critiques of the pieces she commissioned.[2] She was especially involved in the artistic process of her own portraits.[3] Isabella was specific and intentional about her portrayal in these images, as she intended to, art historian David Alan Brown writes, "be portrayed in what she . . . regarded as an appropriately classical manner, befitting her dignity as a ruler."[4] This paper examines the portrait drawing of Isabella created by Leonardo da Vinci and compares it to other portraits of her created by Gian Cristoforo Romano, Francesco Francia, and Titian. These depictions of Isabella are analyzed for the way they convey her education, cultural influence, and political station as she emulated traditionally masculine elements of portraiture in her calculated self-portrayal to achieve greater power and influence at court

Isabella Commissions Leonardo da Vinci

Isabella was first interested in commissioning a portrait from Leonardo da Vinci when she saw a piece he had painted of Cecilia Gallerani, a lady from the court at Milan. An exchange of letters between the marchesa and Cecilia in 1498 conveyed Isabella's desire to view the painting.[5] She wished to compare it to works in her possession by Giovanni Bellini, from whom she had previously commissioned pieces.[6] Leonardo's portrait of Cecilia, which today

1 Sarah Cockram, *Isabella d'Este and Francesco Gonzaga: Power Sharing at the Italian Renaissance Court*. (Farnham: Routledge, 2013), 6–8.

2 Rose Marie San Juan, "The Court Lady's Dilemma: Isabella D'Este and Art Collecting in the Renaissance." *Oxford Art Journal* 14, no. 1 (1991): 68. JSTOR.

3 *Ibid*., 71.

4 David Alan Brown. "Leonardo and the Ladies with the Ermine and the Book." *Artibus Et Historiae* 11, no. 22 (1990): 59. JSTOR.

5 *Ibid*., 50.

6 J. M. Fletcher. "Isabella D'Este and Giovanni Bellini's 'Presepio.'" *The Burlington Magazine 113*, no. 825 (Decem-

Figure 1: Leonardo da Vinci*, Lady with an Ermine,* c. 1489–90, oil on wood panel, 54 cm. x 39 cm. National Musem in Krakow. Sourced from Wikimedia Commons. This image is in the public domain.

is known as *Lady with an Ermine (Figure 1)*, was famous among Italian nobility for the beauty of the sitter and Leonardo's innovative and expressive portrayal of her.[7] Cecilia is portrayed in a three-quarter view—defined as a pose at an angle between frontal facing and side profile — with great attention paid to the portrayal of her expression and pose, seen in the twist of her torso as she gazes over her shoulder and the gentle caress of her hand on the back of the ermine.[8] Isabella requested to borrow the piece to conduct a *paragone*—an artistic comparison—to other art in her collection. This proposed *paragone* might have been a simple pretense as the marchesa may have intended to use the portrait for a much greater comparison, employing it as an inspiration for her own portraiture.[9] A different portrait by the Mantuan court painter Lorenzo Costa is believed by some scholars to depict Isabella d'Este *(Figure 2)*. In a comparison of Costa's portrait *(Figure 2)* to *Leonardo's Lady with an Ermine (Figure 1)*, one can see how artists and patrons of the Mantuan court were interested in evoking the style of an artist as renowned in this period as Leonardo, emulating specific characteristics of other portraits in their own. Isabella would go on to emulate specific aspects of the portraiture of male rulers in some of her portraits.

ber 1971): 703. JSTOR.

7 Brown, "Leonardo and the Ladies," 50.

8 *Ibid.*, 52–53.

9 Francis Ames-Lewis, I*sabella and Leonardo: The Artistic Relationship Between Isabella d'Este and Leonardo da Vinci*, 1500–06 (New Haven: Yale University Press, 2012), 218.

Previous to this exchange of letters and art with Cecilia Gallerani, Isabella had commissioned a portrait by the primary painter of the Mantuan court, Mantegna, and another by Giovanni Santi of Urbino. But she was dissatisfied with both portrayals, claiming neither captured her likeness.[10] Her critique of these works reveals her astute awareness of the importance of her representation in these images, especially how they would affect her status at court. She may have not only been concerned with a portrait that conveyed a flattering physical resemblance, but of one that represented qualities of her personal character and status.[11] In 1499, shortly after viewing Leonardo's painting of Cecilia Gallerani, Lady with the Ermine, Isabella invited Leonardo to Mantua. While living and working as a painter for the Mantuan court, Leonardo made a drawing of Isabella that was intended to act as a preparatory drawing for a painting. However, the painting was never executed by the master artist since his other commissioned work and special interests occupied him elsewhere.[12] The surviving drawing *(Figure 3)* is now housed at the Louvre in Paris, and a second drawing *(Figure 4)*, a copy done by one of Leonardo's assistants, is housed at the Ashmolean Museum at Oxford. The following discussion of Leonardo's work will focus primarily on examining the Louvre drawing.

Figure 2: Lorenzo Costa*, Portrait of a Lady with a Lap Dog* (possibly Isabella d'Este), c. 1500–05, oil on wood panel, 45.4 cm. x 35 cm. Sourced from Wikimedia Commons. This image is in the public domain.

10 *Ibid.*, 53.

11 Sally Hickson. "'To See Ourselves as Others See Us': Giovanni Francesco Zaninello of Ferrara and the Portrait of Isabella d'Este by Francesco Francia." *Renaissance Studies* 23 (June 2009): 290. Wiley Online Library.

12 Ames-Lewis, *Isabella and Leonardo*, 116–17: 218.

Figure 3: Leonardo da Vinci, *Portrait Drawing of Isabella d'Este,*1499, chalk on paper, 61 cm. x 48.4 cm. Paris, Louvre. Sourced from Wikimedia Commons. This image is in the public domain.

Figure 4: After Leonardo da Vinci, *Portrait Drawing of Isabella d'Este,* 1467–1519, chalk on paper, 62.9 cm. x 48.4 cm. Ashmolean Museum, Oxford. Sourced from Wikimedia Commons. This image is in the public domain.

The Drawing: Leonardo's Portrayal of Isabella

Many elements in Leonardo's portrayal of Isabella are significant, for this drawing *(Figure 3)* exemplifies her status and portrays her in traditionally masculine modes. The first element to note is the finish of the drawing. The portrait was created using black chalk applied to paper with added yellow coloration for her dress and red for the hair and skin.[13] Leonardo paid careful attention to the modeling of her face and detailed depiction of her dress,[14] adding stripes decorating the bodice and billowing, flounced bows at the sleeves. Art historian Francis Ames-Lewis writes in his examination of the portrait that it "is perhaps the most sophisticated and finely finished example of the genre of autonomous portrait drawing that evolved in northern Italy towards the end of the fifteenth century."[15] The extensive care put into each aspect of the drawing reveals how every detail was important to the portrayal of not just Isabella's appearance, but also her political station and social status.

Another significant aspect of Leonardo's drawing of the marchesa is the scale of the drawing and the size of the intended painting. The drawing and commissioned painting were unusually large compared to other female portraits at the time, including the Lady with an Ermine. Isabella's portrait would be much more monumental than the portrait of a typical high status lady of the Italian court. The scale of her portrait would be more similar to the size of portraits depicting male rulers of the late fifteenth century.[16] Therefore, the size of the drawing and intended image would equate the marchesa's status with that of ruling men of the northern Italian courts.

The pose of the sitter is also instrumental in successfully representing Isabella's status. Unlike the portrait of Cecilia, the marchesa is depicted in a strict profile pose with a front-facing torso. The formality of this posture appears to be a reversion from Leonardo's development of expressive gesture in his previous portraiture—after all, he was renowned for the way he captured momentary expressions thought to be revealing of the sitter's personality.[17] While Lady with an Ermine represents the sitter in a very contemporary style, focusing on highlighting her beauty and femininity, the drawing of Isabella draws on a more formal and traditional style associated with male rulers of Italy's ancient Roman past, which was emulated by the male rulers of the Renaissance period. Scholars, such as David Alan Brown and Ames-Lewis, have proposed that Leonardo portrayed her in this much more formal pose at

13 Brown, "Leonardo and the Ladies," 54.
14 *Ibid.*, 57.
15 Ames-Lewis, *Isabella and Leonardo*, 125.
16 Ames-Lewis, *Isabella and Leonardo*, 119; Brown, "Leonardo and the Ladies," 54.
17 Brown, "Leonardo and the Ladies," 58–60; Ames-Lewis, *Isabella and Leonardo*, 161.

her request,[18] and it is possible she wished to emulate the masculine portraiture and associated status of her ruling male contemporaries. Many scholars also theorize Leonardo's portrait drawing of Isabella was directly based off of an earlier portrait of the marchesa on the Romano medal, which similarly portrays her in a traditionally formal manner.

Romano and Leonardo: Portraits of Classical Male Power

The medallic portrait of the marchesa designed by sculptor Gian Cristoforo Romano *(Figure 5)* portrays Isabella in a classical manner to evoke the antique and assert her high status. The design of Romano's medal was based on medallions and coins from Classical Antiquity portraying Roman

Figure 5: Gian Christoforo Romano, *Cast bronze medal of Isabella d'Este, Marchioness of Mantua,* 1498, cast bronze, diameter 38.5 mm. London, British Museum. This image is licensed under CC BY-NC-SA 4.0.

Figure 6: *Roman Imperial Medallion depicting Nero,* 64 CE, cast copper alloy. London, British Museum. bronze, diameter 38.5 mm. London, British Museum. This image is licensed under CC BY-NC-SA 4.0.

emperors. Classical Antiquity describes the ancient past of Greco-Roman culture, a period that fascinated artists and scholars during the Italian Renaissance and influenced much of their work. Isabella was known to collect antique Roman medallions, adding them to her prolific collection of antique and contemporary art. She commissioned the Romano medal to commemorate her image in a manner reflective of her high status

18 Ames-Lewis, I*sabella and Leonardo*, 160; Brown, "Leonardo and the Ladies," 60.

as a ruler.[19]

Antique rulers, such as first-century Roman Emperor Nero, were venerated with depictions of their image in cast-metal, often in the form of commemorative medallions or coins *(Figure 6)*. In these antique portraits, the rulers' heads were depicted in a linear profile, their strict pose meant to display their strength and power.

Leonardo's portrait shows Isabella in a controlled pose meant to convey her vitality and authority, emulating the perceived strength and power of the Roman rulers represented in their antique medallic portraits. In fact, Leonardo presents her even more formally than Romano did on her medal.[20] Leonardo altered the depiction of her hair from loosely pulled back in a flowing updo, as seen on the Romano medal, to neatly falling down her back under a restrictive veil. The direction Isabella is facing on the Romano medal and in Leonardo's drawing is also highly significant: Almost all fifteenth-century Italian portraits of women in profile were shown facing the left,[21] and men were typically shown facing the right. By depicting her in profile facing the right, Leonardo references both the medal and painted portraits of men of a status the marchesa wished to emulate.

The Marchesa's Cultural Authority Displayed

Leonardo and Romano not only portrayed Isabella in a classical mode to equate her status to that of antique and contemporary male rulers, but they also did so to reference her extensive knowledge of antiquity. Knowledge of the Classical Antique was highly valued by Italian nobility during the Renaissance period as they believed classical Greco-Roman history represented preeminent cultural authority and political leadership. They regularly emulated classical art to relate themselves to the Roman leaders. As the daughter of the duke and duchess of Ferrara, Isabella was raised among the Italian elite at the Ferrara court and was exposed to a thorough humanist education, studying Latin and the classical arts.[22] The marchesa, astutely aware of Leonardo's mastery of subtle gesture, originally had the artist depict her with her arms resting atop a parapet, her right hand pointing to a book resting to her left. The book is clearly present in the Ashmolean copy of Leonardo's drawing *(Figure 4)*, and the gesture and traces of the book remain evident in the Louvre drawing as well *(Figure 3)*. This book acts as a representation of her masterful knowledge of the antique as understood by scholars of the Renaissance period. The book symbolizes her studies in the classical tradition as well as her

19 Ames-Lewis, *Isabella and Leonardo,*106.
20 Brown, "Leonardo and the Ladies," 48–49.
21 Ames-Lewis, *Isabella and Leonardo*, 113.
22 San Juan, "The Court Lady's Dilemma," 70.

prolific patronage of poets and court writers.[23] Here, Leonardo shows Isabella literally and metaphorically pointing to her cultural prowess as she associates herself chiefly with her classical education and patronage of the arts, cultural attributes typically only possessed by men of this period.

Isabella's active support of the arts enabled her to strengthen her position at court as she expanded her influence to include curating cultural taste. Her art patronage gained her positive social exposure to elite men of intellectual and artistic spheres, simultaneously strengthening the cultural and moral authority of the ruling family and bolstering her own reputation. The marchesa was renowned for her prolific collection of art and artifacts, which she displayed in the *studiolo* and *grotta* of her private apartments at the Mantuan palace[24]. *Studiolo* is the Italian term for a small, elaborately decorated room that functioned as a gallery and a space for studying, reading, and writing. Isabella's *grotta*, a vaulted ground-floor room, was located directly below her *studiolo* and was similarly ornamented with pieces from her collection. These private rooms were accessible to the nobility Isabella wished to entertain, and the art adorning their walls would be of great interest to visitors as they were able to admire the work from antique and contemporary artists in person.[25] In the Renaissance period, the collection itself was seen as a direct reflection of its patron's taste and personal character.[26] Isabella was highly regarded in her time for having impeccable taste, and therefore great character.

Stemming from her Latin-based studies, she had a preference for classical and mythological subject matter,[27] which dominated the paintings she collected and displayed in her *studiolo* and *grotta*.[28] This iconographic bias towards the antique was also rooted in Renaissance ideas of cultural ethicality as humanist scholars of this period connected classical ideas of intellect and ethics to moral superiority.[29] By sponsoring the cultivation of art and music in the humanist tradition, Isabella was responsible for establishing herself and her husband as virtuous leaders. The scale of her collection was equal to that of the male members of her family.[30] Yet by establishing her authority in a masculine mode, the marchesa was able to occupy a scholarly male space at the court, emphasizing her social and political eminence. These aspects of Isabella's public personality are indicated in Leonardo's portrait through the

23 Lisa K. Regan. "Ariosto's Threshold Patron: Isabella D'Este in the Orlando Furioso." *Modern Language Notes* 120, no. 1 (January 2005): 50–53. JSTOR.
24 San Juan, "The Court Lady's Dilemma," 69–71.
25 *Ibid.*, 67.
26 *Ibid.*, 75.
27 *Ibid.*
28 Stephen J. Campbell. *The Cabinet of Eros: Renaissance Mythological Painting and the Studiolo of Isabella d'Este.* (New Haven: Yale University Press, 2006), 64–65.
29 San Juan, "The Court Lady's Dilemma," 69.
30 *Ibid.*, 71.

inclusion of the book with her simple gesture emphasizing it, a significant act of self-portrayal.

Isabella's Participation in the Image-making Process

Despite Leonardo's failure to produce a complete painted version of the portrait, the drawing was most likely used as a reference for many of her portrait commissions that followed, such as one completed by Francesco Francia in 1511. The marchesa was unable to sit for the painter, so Francia had to rely exclusively on previously illustrated depictions of Isabella and written descriptions of her.[31] Due to the fact that there are no surviving copies of the Francesco Francia portrait, there is no way to properly compare Francesco's depiction of Isabella to that of Leonardo's drawing.[32] Therefore, the following discussion of this work will focus on how Isabella used this portrait to fashion and propagate her social identity as it relates to the discussion of her use of portraiture as a tool to facilitate power.

Isabella was very involved in the art making process of her commissions. This is demonstrated by her correspondence with artists, such as her four-year negotiation over an *istoria* painting she also commissioned from Francesco Francia. An *istoria* is a type of narrative and allegorical scene derived from antique and Christian texts. Paintings featuring this subject matter were a favorite of Renaissance-period patrons, including Isabella. This negotiation in which Isabella asserted her preferences of subject matter and art style[33] exemplifies her unrelenting vocalization of artistic opinions when working with an artist. This critique of her employees' work is most prominent in her appraisal of the portraits she commissioned, such as the work painted by Francesco Francia. Before commissioning her own portrait from the artist, she requested a portrait of her son, Federico Gonzaga II *(Figure 7)*. The portrait of the young boy was completed quickly and delivered to Isabella. She was satisfied with the image, except for the color of her son's hair. She is reported to have given the portrait back to Francia, and he corrected the coloration at the marchesa's behest.[34]

Shortly after the portrait of her son was complete, Isabella commissioned Francia to create a portrait of herself in 1511. She was involved in the image-making process for this portrait, too. She not only sent the main reference image on which to base the portrait—a painting of her by Lorenzo Costa which was reportedly her favorite portrait—but she also had a relative

31 Hickson, "'To See Ourselves as Others See Us,'" 295–97.
32 *Ibid*., 297.
33 Fletcher, "Isabella d'Este and Giovanni Bellini's 'Presepio,'" 711.
34 Hickson, "'To See Ourselves as Others See Us,'" 295.

Figure 7: Francesco Francia, *Portrait of Federico Gonzaga,*1510 tempera on wood, 45.1 c.m. x 34.3 c.m. New York, Metropolitan Museum of Art. This image is in the public domain.

and close acquaintance oversee the process on her behalf.[35] Upon receiving the portrait, Isabella wrote to the artist, claiming he had portrayed her in a manner "far more beautiful by your art than nature," which reveals she was acutely aware the portrait portrayed her in an idealized manner.[36] The marchesa herself played an active role in the crafting of this image, which did not accurately represent her physical appearance but rather properly portrayed her high status and emphasized her virtuous personality by representing her likeness in an idealized and specifically classicizing manner.

35 *Ibid.*, 295–97.
36 *Ibid.*, 297.

Isabella Shares her Image at Court

After the marchesa negotiated the crafting a flattering portrayal that conveyed her superior character and high rank, she disseminated the portrait among courtiers to share her self-representation. As art historian Sally Hickson states in her examination of the oral discourse surrounding the marchesa's portraiture, "[Isabella's] chief arena for staging her public persona was through the cultivation of her image in portraits given as gifts and widely circulated among her contemporaries."[37] For example, the marchesa gifted the Francia portrait to Gian Francesco Zaninello, a courtier and poet from Ferrara who displayed the painting at multiple dinner parties he hosted. At these dinners, elite members of the northern Italian court viewed Isabella's self-fashioned portrayal, engaged with its content, and discussed the beauty and merit of the sitter.[38] Another way she shared her image at court was through the dissemination of the Romano medal, which was previously discussed. The marchesa had many copies of the Romano medal cast in bronze, and she gifted these medallic portraits to members of different Italian courts. This circulated her image among the elite, spreading a likeness of her associated with the antique and famed rulers. The Latin motto inscribed on the medal translated reads: "on account of high merits," which is reflective of both the high merit of the gift's recipient who had gained Isabella's favor and that of the woman portrayed on its surface. By sharing these images herself, which portray her idealized likeness in classicizing and masculine modes that attested to her cultural and political power, Isabella was able to control the way she was perceived by the Mantuan court and assert herself as an independent leader.

The Marchesa's Application of her Power

The power the images gained Isabella was not solely symbolic. With the elevation of her status, due in great part to the cultivation of her public image through portraiture, the marchesa was able to assert real authority over Mantuan politics. Italian Renaissance scholar Sarah Cockram writes in her research on Isabella's co-rule of Mantua, "as consort, Isabella was cast as the junior partner in the couple's team dynamic . . . However, while carefully maintaining an unblemished reputation for virtue and feminine accomplishment, the marchesa encouraged praise of her virile, princely qualities—validating her entry into male-dominated cultural spheres, and politics."[39] Isabella had become the official consort of Mantua at age 16 and maintained her indepen-

37 *Ibid*,. 291–92.
38 *Ibid*., 300–301.
39 Cockram, *Power Sharing,* 7.

dence by managing her own finances and court. Since her husband was often away from court on military campaigns, Isabella assumed the responsibilities and authority of the ruler in his stead.[40] This position would have typically been taken over by the next most powerful man at court, Francesco Secco, Mantua's lieutenant general of state.[41] But the marchese made explicit in his correspondence he wished for Secco to be superseded by his wife in matters of the state in his absence.[42]

The period during which Isabella and Francesco ruled was full of political turmoil and conflict as they worked together to forge alliances with neighboring Italian states. In 1491, Isabella herself traveled to Milan to negotiate peace and strengthen Mantuan relations with the Milanese ruler Lodovico Sforza.[43] Eighteen years later, she would be instrumental in the negotiations surrounding the liberation of her husband after the marchese was taken prisoner by the Venetians during the War of the League of Cambrai. Isabella served as regent during the eleven-month period her husband was captured.[44] The marchese and the Mantuan nobility's clear trust in the marchesa's political leadership was fully realized when she was left to rule after Francesco's death, acting as regent until her son was old enough to assume the head of state's responsibilities. She then successfully established herself as sole ruler of Solarolo, a small region in the province of Ravenna that she ruled as a city-state for the last decade of her life.[45]

The authority Isabella asserted did not go unchallenged, though, as the social position of consort was limited by fifteenth century expectations for women to remain subordinate. Isabella's contemporaries expected her to be more concerned with social and familial matters[46] rather than the state politics in which she was so actively involved. Additionally, there was an immense pressure placed on the marchesa to provide Francesco with an heir. Her position at court was not fully secure until 1500 when she finally gave birth to her son.[47] The marchesa's financial and political independence from her husband was uncommon for this period, and her co-rule of the state was often challenged by powerful courtiers.[48] Cockram writes, "Isabella aimed to possess influence and fame without arousing opposition in an age antagonistic to unambiguously dominant women, even consorts. Thus, she deployed conscious self-fashioning and careful image management to direct her posi-

40 *Ibid.*, 18.
41 *Ibid.*, 87.
42 *Ibid.*, 89.
43 *Ibid.*, 20.
44 *Ibid.*, 162–64.
45 *Ibid.*
46 *Ibid.*, 4–5.
47 *Ibid.*, 22.
48 *Ibid.*, 28.

tion within the restrictions placed on the Italian Renaissance noblewoman."[49] Isabella was able to assert her political authority, successfully ruling alongside and independent of her husband due to her ability to subtly manipulate and propagate a specific image of herself at court. The marchesa subtly portrayed her "virile" and "princely" qualities[50] through the masculine-encoded aspects of the portraiture previously discussed while at the same time maintaining her reputation as an elite woman by performing female virtue through her displays of femininity in portraiture.

A Display of Female Power: Titian's Portrayal of Isabella

Another portrait of the marchesa, done by Titian in 1536—often referred to as Isabella in Black *(Figure 8)*—is an excellent example of the marchesa's more feminine-type portraiture. This painting was based on previous portraits done of Isabella by Costa, Francia, and Leonardo; yet it differs greatly in its representation of the marchesa as her depiction is much more similar to Leonardo's painting of the *Lady with an Ermine* [51] in the way it emphasizes the sitter's poised beauty in traditionally feminine modes. Titian's depiction of Isabella differs from Leonardo's drawing of her in that Titian shows the marchesa in a three-quarter view, her left side presented to the viewer. As previously discussed, Leonardo's portrait portrayed Isabella in a right-facing profile, emulating the pose of antique rulers on Roman medallions. Titian employs this different pose to emphasize the sitter's beauty in an idealized manner. Here, her status is represented not by her pose or the inclusion of a symbolic object, but by her elaborate attire as Titian paid careful attention to painting the ornate details of her headdress, the brocade fabric of her clothes, and the fur draped over her shoulder. Art historian David Alan Brown maintains this image captures the marchesa's "vitality and uniqueness as an individual."[52] At the time of its creation, Isabella was over 60 years old, but she saw to it Titian painted her as a young woman in the portrait.[53] The marchesa is shown in what was culturally recognized as the prime of her life and height of her beauty in order to emphasize her physical beauty.

Titian represents Isabella in a mode much more characteristic of female portraiture, signifying her attractiveness, social rank, and virtuous chastity.[54] Her portrayal in the Titian portrait, as well as the Lorenzo Costa painting previously discussed, maintains her femininity, asserting her social status

49 *Ibid.*, 4–5.
50 *Ibid.*, 7.
51 Brown, "Leonardo and the Ladies," 59–60; Hickson, "'To See Ourselves as Others See Us,'" 305.
52 Brown, "Leonardo and the Ladies," 60.
53 Hickson, "'To See Ourselves as Others See Us,'" 305.
54 San Juan, "The Court Lady's Dilemma," 74.

Figure 8: Titian, *Isabella in Black,* 1536 oil on canvas, 102 c.m. x 64 c.m. Kunsthistorisches Museum. Image sourced from Wikimedia Commons. This image is in the public domain.

through its portrayal of her virtue and beauty, rather than rank—as represented in her earlier portraiture. The combination of this feminine portrayal with her more masculine-encoded portraiture established Isabella as both a *woman* of high status and a *ruler* of high rank. Through her art patronage, she was able to transverse into male-exclusive realms of the court and transcend the gendered roles of the ruling class all while maintaining her status as an elite woman.

Conclusion

Often heralded as the "First Lady of the Renaissance," Isabella d'Este was well integrated into the politics and social life of the fifteenth century Italian courts. She was held in high regard by members of the nobility for her cultural trend-setting, intellectual prowess, patronage of the arts, and political leadership during the height of the Gonzaga family's reign in Mantua. The marchesa's esteemed reputation was no simple coincidence, but as proven through the examination of the many portraits she commissioned, was a result of her thoughtful and active participation in crafting flattering and authoritative images of herself. These portraits, fashioned over the span of her life and the course of her political career, asserted her as a powerful and cultured individual, as seen in the Romano medal and Leonardo drawing, while retaining her femininity and social status through idealized portrayals of her beauty, evident in the works of Costa Lorenzo, Francesco Francia, and Titian. Over the course of her public career as Marchesa of Mantua, Isabella successfully entered masculine spheres of patronship and leadership. Crossing into these male-dominated areas of court life was facilitated by her self-presentation through portraiture in traditionally masculine modes, asserting herself as an equal in status and rank to men of the ruling class. Thus, Isabella proved to not only possess a great appreciation for art, but also a clear understanding of its power to transform herself and transcend the limits of the world around her.

Bibliography

Ames-Lewis, Francis. *Isabella and Leonardo: The Artistic Relationship Between Isabella d'Este and Leonardo da Vinci*, 1500-1506. New Haven: Yale University Press, 2012.

Brown, David Alan. "Leonardo and the Ladies with the Ermine and the Book." *Artibus Et Historiae* 11, no. 22 (1990): 47–61. JSTOR, https://www.jstor.org/stable/1483398.

Campbell, Stephen J. *The Cabinet of Eros: Renaissance Mythological Painting and the Studiolo of Isabella d'Este*. New Haven: Yale University Press, 2006.

Cockram, Sarah. *Isabella d'Este and Francesco Gonzaga: Power Sharing at the Italian Renaissance Court*. Burlington, Farnham: Routledge, 2013. EBSCOhost eBook Collection, https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=nlebk&AN=573163&site=eds-live&scope=site&custid=039-820.

Fletcher, J. M. "Isabella d'Este and Giovanni Bellini's 'Presepio.'" *The Burlington Magazine* 113, no. 825 (December 1971): 703–13. JSTOR, https://www.jstor.org/stable/876808.

Hickson, Sally. "'To See Ourselves as Others See Us': Giovanni Francesco Zaninello of Ferrara and the Portrait of Isabella d'Este by Francesco Francia." *Renaissance Studies* 23 (June 2009): 288–310. Wiley Online Library, https://doi.org/10.1111/j.1477-4658.2009.00565.x.

Regan, Lisa K. "Ariosto's Threshold Patron: Isabella d'Este in the 'Orlando Furioso.'" *Modern Language Notes* 120, no. 1 (January 2005): 50–69. JSTOR, http://www.jstor.org/stable/3252027.

San Juan, Rose Marie. "The Court Lady's Dilemma: Isabella D'Este and Art Collecting in the Renaissance." *Oxford Art Journal* 14, no. 1 (1991): 67–78. JSTOR, https://www.jstor.org/stable/1360278.

Figure Credits

Figure 1: Leonardo da Vinci, *Lady with an Ermine,* c. 1489–90, oil on wood panel, 54 cm. x 39 cm. National Musem in Krakow. Sourced from Wikimedia Commons. This image is in the public domain. https://commons.m.wikimedia.org/wiki/File:Leonardo_da_Vinci_(1452-1519)_-_Lady_with_an_Ermine_%E2%80%93_Portrait_of_Cecilia_Gallerani_(ca._1473%E2%80%931536)_-_MNK_XII-209_-_National_Museum_Krak%C3%B3w.jpg.

Figure 2: Lorenzo Costa, *Portrait of a Lady with a Lap Dog* (possibly Isabella d'Este), c. 1500–05, oil on wood panel, 45.4 cm. x 35 cm. Sourced from Wikimedia Commons. This image is in the public domain. https://commons.wikimedia.org/wiki/File:Lorenzo_Costa_(1460-1535)_-_Portrait_of_a_Lady_with_a_Lapdog_-_RCIN_405762_-_Royal_Collection.jpg.

Figure 3: Leonardo da Vinci, *Portrait Drawing of Isabella d'Este,*1499, chalk on paper, 61 cm. x 48.4 cm. Paris, Louvre. Sourced from Wikimedia Commons. This image is in the public domain. https://commons.wikimedia.org/wiki/File:Leonardo_da_Vinci_-_Portrait_d%27Isabelle_d%27Este,_MI_753,_Recto.jpg.

Figure 4: After Leonardo da Vinci, *Portrait Drawing of Isabella d'Este,* 1467–1519, chalk on paper, 62.9 cm. x 48.4 cm. Ashmolean Museum, Oxford. Sourced from Wikimedia Commons. This image is in the public domain. https://commons.wikimedia.org/wiki/File:D%27apr%C3%A8s_L%C3%A9onard_de_Vinci,_Portrait_d%27Isabelle_d%27Este,_XVIe_si%C3%A8cle,_Oxford,_Ashmolean_museum.jpg.

Figure 5: Gian Christoforo Romano, *Cast bronze medal of Isabella d'Este, Marchioness of Mantua,* 1498, cast bronze, diameter 38.5 mm. London, British Museum. This image is licensed under CC BY-NC-SA 4.0. https://www.britishmuseum.org/collection/object/C_M-8.

Figure 6: *Roman Imperial Medallion depicting Nero,* 64 CE, cast copper alloy. London, British Museum. bronze, diameter 38.5 mm. London, British Museum. This image is licensed under CC BY-NC-SA 4.0. https://www.britishmuseum.org/collection/object/C_R-10007.

Figure 7: Francesco Francia, *Portrait of Federico Gonzaga,*1510 tempera on wood, 45.1 c.m. x 34.3 c.m. New York, Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/436333.

Figure 8: Titian, *Isabella in Black,* 1536 oil on canvas, 102 c.m. x 64 c.m. Kunsthistorisches Museum. Image sourced from Wikimedia Commons. This image is in the public domain. https://commons.wikimedia.org/wiki/File:Titian_-_Isabella_d%27Este,_Duchess_of_Mantua_-_WGA22921.jpg.

College of Humanities and Public Affairs

Negotiating the Gendered Powers of the Dead: An Exploration of Identity and the Afterlife through Funerary Masks and Coffins

Hannah Fuller

Abstract

Ancient Egyptian burial and the artifacts that accompanied it was an intricate process that sought to commemorate the deceased's mortal life while properly equipping them to thrive in the hereafter. Functionally, the funerary coffin and funerary mask acted as the protective container of the deceased, but ritualistically it served a much greater purpose. These artifacts not only provided the material manifestation of memorializing the deceased's life, but they simultaneously transfigured the deceased for their ideal state in the hereafter. Manifesting resources, protection, and even divine status, the funerary mask and coffin acted as an intensely personal reflection of the individual's idealized self while also invoking the religious powers needed to transgress to the divine plane. At the culmination of self, transformation, and death, the funerary mask and coffin offer a compelling point of analysis at the intersection between identity and funerary practice. These aspects are further complicated when gender, a vital component in identity and the ancient Egyptian notion of divine corporal ascension, is incorporated as an aspect of study. With these distinctions in mind, the funerary mask and coffin acts as a catalyst for understanding ancient Egyptian conceptions of gender, identity, and death.

Introduction

Mortuary practice in ancient Egypt was a multifaceted process that used texts, artifacts, and ritual in the ceremonial parting with the deceased. Egyptian funerary customs are unique in that they not only acted as a method of eternally memorializing the dead, but they also transformed a body for a successful transition into the afterlife. While multiple pieces of funerary equipment were used in this process, the funerary mask and coffin are of particular interest as they act as the physical intersection between the deceased's memorialized identity and the transformational process. Another layer of complexity is added when aspects of the deceased's identity, such as gender, influence memoriaization land transformative properties of the funerary mask and coffin. Through an analysis of funerary masks and coffins, it becomes clear that transformation to the afterlife took precedence over a realistic memorialization of the individual, resulting in a minimization of highly gendered aspects of funerary equipment.

Material Composition and Practical Function

Before the symbolic elements of funerary masks and coffins can be realized, their material composition must first be examined in order to understand their larger purpose. To properly survey the material of these objects, it must also be asserted that the funerary equipment enclosing the dead saw multiple shifts throughout Egyptian history, with certain trends and materials falling in and out of popular use. Coffins and masks emerged as an element of funerary practice early in Egyptian history, coming into use in the Old Kingdom. Helmet-masks were fitted over the head and shoulders of the mummy, and the body itself was placed within a wooden, rectangular coffin, serving as a protective replacement for previous methods that covered the entirety of the bandaged body in plaster.[1] During the Twelfth Dynasty, anthropoid coffins emerged as an extension of the funerary mask, creating a casing that aimed to fully embody the deceased individual it enclosed.[2] Coffin styles and the use of funerary masks saw multiple innovations throughout ancient Egypt but generally remained as a consistent feature of burials from their initial inception in the Old Kingdom. The Eighteenth Dynasty saw an increased popularity in the use of masks and anthropoid coffins among both elites and common people,[3] and this practice persisted well into the Roman period. As the afterlife became more and more accessible, individual, familial tombs declined in

1 Salima Ikram and Aidan Dodson, *The Mummy in Ancient Egypt: Equipping the Dead for Eternity* (London: Thames and Hudson, 1998), 29.
2 *Ibid.*, 34.
3 *Ibid*, 38–40.

favor of mass tombs; coffins in later periods became a necessity for individual expression and burial.[4] For the purposes of this exploration, masks and coffins from the New Kingdom onward will be the focus of study due to their more individualistic style following the implementation of the anthropoid coffin in the Middle Kingdom.

The decorative and often fragile construction of funerary masks and coffins reveals their purpose as both ritualistic and personal. Masks and anthropoid coffins were most commonly made from cartonnage, a material created from layering linen or papyrus with plaster or resin and then shaping it around a mold to form a silhouette that resembled the body of the deceased.[5] The cartonnage casing would then be adorned with paint to imbue the mask and coffin with decorative elements and religious symbolism, such as spells and symbols, invoking divine protections.[6] Paint acted as an accessible means of emulating the appearance of more precious materials; however, if the individual could afford it, coffins and masks incorporated expensive elements such as gilding and inlaid stone.[7] Although other materials such as stone and wood were common in the construction, the commonality of cartonnage and the decorative elements that adorned the equipment clearly show these items were not highly durable and were meant to remain eternally undisturbed following their entombment. This is reflective of the private intent of the funerary mask and coffin, making clear the personal aspects and transcendent properties tied to these objects simply in the very act of their creation.

Appropriating Identity: The Complex Function of Memorialization

Coffins and funerary masks were not only protecting the body they enclosed but were also an important means of directly memorializing the image of the deceased individual. While masks and coffins were highly idealized representations of the deceased, the depiction of idealized characteristics[8] still reflect a sense of individual identity, or at least the identity individuals sought to display. One of the most common features of representation and appropriation prevalent in these items was the intent to preserve an identity of elite

4 Kathlyn M. Cooney, "Changing Burial Practices at the End of the New Kingdom: Defensive Adaptations in Tomb Commissions, Coffin Commissions, Coffin Decoration, and Mummification," *Journal of the American Research in Egypt* 47 (2011): 20. JSTOR.

5 John Baines and Jaromir Malek, *Cultural Atlas of Ancient Egypt Revised Edition* (New York: Checkmark Books, 2000): 221.

6 Cooney, "Changing Burial Practices," 29.

7 *Ibid.*, 28.

8 Christina Riggs, "Facing the Dead: Recent Research on the Funerary Art of Ptolemaic and Roman Egypt," A*merican Journal of Archaeology* 106, no. 1 (2002): 95. JSTOR.

status and wealth.[9] As previously stated, paint was a primary way to give the appearance of rare materials such as gold and gemstones as a means of presenting access to wealth. Golden coloring displayed not only an image of elite status due to material access, but more importantly, it directly portrayed the deceased individual as a divine being as the gods were believed to have skin of gold.[10]

Figure 1: *Mummy of Khnumhotep with mask and broad collar.* Metropolitan Museum of Art. This image is in the public domain.

This same kind of status emulation was also accomplished through the employment of royal iconography, such as the mask of Khumenhotep, whose mask features a uraeus and false beard, despite never acting as a king in life (*Figure 1*). Less extreme co-opting was also prevalent, typically in the form of painted representations of earrings and jewelry on masks, again giving the appearance of being able to afford such pieces regardless of if an individual could do so in reality.[11] Regardless of the reliability of these presentations of wealth, the incorporation of these elements into the funerary masks and coffins are reflective of the ways in which personal identity was distinctly tied to the equipment itself.

With this notion in mind, funerary masks and coffins did include elements that asserted gendered aspects of the deceased individual. Gendered features of the deceased are expressed through funerary equipment but not as consistently nor as explicitly as other identity markers. Funerary masks in particular often lack definable gendered features, and many are left unidentifiable, especially when excavated without the accompanying context of a coffin or a tomb. The lack of definable gendered aspects serves a functional purpose in the figurative transformation of the deceased that requires the implementation of masculine and feminine powers.[12] Regardless of the ambiguities that accompany asserting gender through funerary equipment alone, masks and

9 *Ibid.*, 99.

10 Sidney M. Goldstein, "Egyptian and Near Eastern Art," *Bulletin (St. Louis Art Museum)* 19, no.4 (1990): 14. JSTOR.

11 Christina Riggs, "Roman Period Mummy Masks from Deir El-Bahri," *The Journal of Egyptian Archaeology* 86 (2000): 128. JSTOR.

12 Kathlyn M. Cooney, "Gender Transformation in Death: A Case Study of Coffins from Ramesside Period Egypt," *Near Eastern Archaeology* 73, no.4 (2010): 236.

coffins still employ decisive indicators of gender. The inconsistencies in gendered depictions make the instances in which femininity and masculinity are explicitly employed that much more noteworthy.

The most common denotations of gendered displayed on coffins and funerary masks include, but are not limited to, facial hair, gendered wigs, jewelry, and anatomical molding such as breasts or rounded facial features.[13] It is important to note the majority of these gendered features were used by both men and women. For instance, many female anthropoid coffins do not have breasts, and male funerary masks will often depict jewelry, an accessory more typical of the female elite. Regardless of who depicted what, these are markers that decisively represent what are seen as gendered aspects of identity. The ways in which the gender of the individual is incorporated into funerary equipment is well represented in the masks of an Eighteenth Dynasty overseer of builders, Amenhotep (*Figure 2*) and his wife, Mutresti (*Figure 3*). This particular set of masks are useful in the comparison of their gendered principles because they have been commissioned by the same family and therefore do not present variation in cultural background or wealth.

Figure 2: *Funerary Mask of the Overseer of Builders Amenhotep.* Metropolitan Museum of Art. This image is in the public domain.

Figure 3: *Funerary Mask of a Woman.* Metropolitan Museum of Art. This image is in the public domain.

13 Ikram and Dodson, *The Mummy in Ancient Egypt*, 169.

Each mask displays gilded skin and inlaid stone, emphasizing their elite status in addition to other markers of wealth, such as Mutresti's elaborate ball beads and Amenhotep's blue and gold striped lappet wig, perhaps alluding to the pattern of the nemes headdress. Despite their shared characteristics, each is clearly gendered. The mask of Mutresti especially so, "[wearing] not the traditional striped headdress and the funerary broad collar, but a wig and jewelry of the most up-to-date designs, seen on the ladies of the court only from the second half of the Eighteenth Dynasty onward."[14] Amenhotep on the other hand, adorns the traditional headdress and no other additional accessories. Each mask presents a variance in the facial structure; Mutresti displays smaller, more rounded features, and Amenhotep's square jaw and large ears are distinct. Although these masks are hardly a universal template for all female and male masks, they are a clear example of the iconography employed when gender is a decisive element of identity included in the design of masks.

Figure 4: *Inner Coffin of the Singer of Amun-Re, Henettaway.* Metropolitan Museum of Art. This image is in the public domain.

Similar gendered expressions are also prevalent, perhaps even more so, in anthropoid coffins. Since the anthropoid coffin is a reflection of the deceased's entire body rather than their face only, it is logical to assume gendered elements are shown in these contexts more explicitly. Through the exam-

14 Nicholas Reeves, "Amenhotep, Overseer of Builders of Amun: An Eighteenth-Dynasty Burial Reassembled," Metropolitan Museum Journal 48, no. 1 (2013): 19.

ination of the inner-coffin of Henettawy (*Figure 4*), a singer of the temple of Amun-Re in the Third Intermediate Period, elements of gendered identity make themselves clear. Most starkly there is the anatomic molding of the coffin shape itself, displaying physical characteristics indicative of a feminine presentation such as a rounded face and narrow waist. In terms of her dress, she dons the female tripartite wig, earrings, and a decorative headband. The sheer space available on the anthropoid coffin, compared to that of a funerary mask, allows for an immense amount of added symbolism in both ritual texts and funerary scenes.

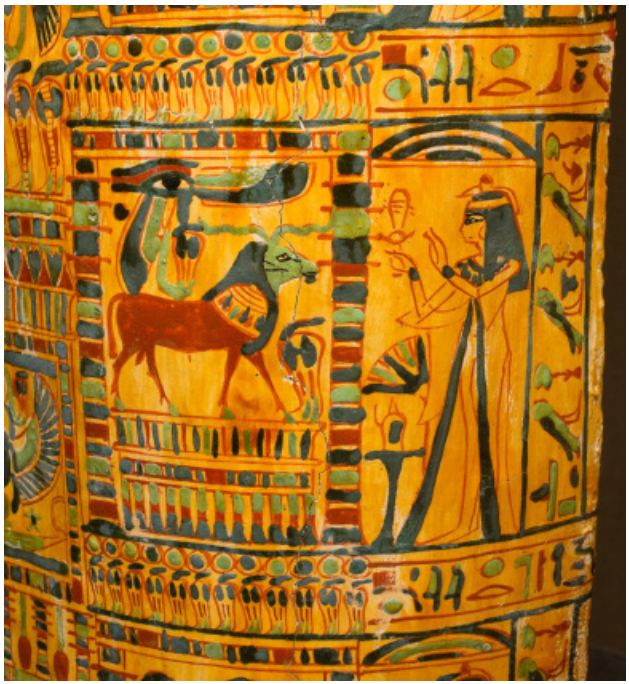


Figure 5: *Inner Coffin of the Singer of Amun-Re, Henettaway.* Metropolitan Museum of Art. This image is in the public domain.

Henettawy's inner coffin is covered in images of the gods, and she depicts herself offering worship to them. (*Figure 5*). In the image of herself in offering, she is displayed in distinctly female artistic conventions such as a white dress[15] and closed stance, an artistic convention employed to depict women as opposed to men, who are often shown in an active stride.[16] The inner coffin of Henettawy is a strong example of the ways in which gendered conventions were incorporated into the coffin display, in both its physical representation of the deceased and the artistic scenes painted upon them that included the individual.

As Henettawy's inner coffin and Amenhotep and Mutresti's masks demonstrate, gendered symbolism was incorporated into the construction of funerary masks and coffins. These gendered depictions served as an indicator of the deceased's own personal identity, along with other markers such as socioeconomic status, that memorialized the ways they sought to be perceived follow-

15 Cooney, "Gender Transformation in Death," 231.
16 Baines and Malek, *Cultural Atlas of Ancient Egypt*, 204.

ing their death. However, it is important to recognize that these examples are not highly indicative of the majority of funerary masks or coffins during this time, rather they represent a small sample of explicitly gendered depictions. In actuality, much of the gendered iconography featured on funerary masks and coffins is either a blend of masculine and feminine symbolism, ungoverned by the gender of the deceased, or not explicit enough to even determine the gender of the individual it represents. With these discrepancies in mind, the symbolic function of gendered symbolism must be analyzed in order to understand its purpose and variation across funerary equipment.

Divine Transformation: The Symbolic Function of Gender

While the identity of an individual was incorporated into the design of funerary masks and coffins, transformative iconography was a more prevalent and significant element of funerary equipment. Divine iconography often subverted explicit gendered elements of the mask and coffin as a means of employing the feminine and masculine properties necessary for transitioning to the afterlife.[17] For the ancient Egyptians, funerary equipment was not simply a means of memorialization, but it rather played an active role in literally transforming the dead to be properly reborn in the afterlife. For instance, including markers of wealth was not simply a means of asserting one was wealthy in life but a means of manifesting those resources in death.

This same concept applies to the divine iconography present on funerary equipment and fulfills an even more vital role in transition to the afterlife. Although aspects of identity were incorporated into the coffin and mask, the primary purpose of iconography em-

Figure 6: *Mummy Mask of Meret-it-es.* Egyptian, Late Period to Ptolemaic Period, 30th Dynasty to Early Ptolemaic Dynasty, ca. 380-250 B.C.E. Cartonnage (linen or papyrus covered in plaster), paint, and gold leaf, 15 1/2 x 9 1/2 x 10 inches (39.37 x 24.13 x 25.4 cm). The Nelson-Atkins Museum of Art, Kansas City, Missouri. Purchase: William Rockhill Nelson Trust (by exchange), 2007.12.3. Photo courtesy Nelson-Atkins Media Services.

17 Cooney, "Gender Transformation in Death," 224.

ployed within the mask and coffins "was not to provide a naturalistic portrait of the deceased but to record his or her assimilation to an Egyptian deity."[18] The dead were unable to reach the afterlife through merely their human form and thus required a literal merging with gods, such as Osiris, whose divine regenerative power allowed for their souls to be reborn into the next world. This metamorphosis was achieved through literal portrayals of the deceased as divine entities and the protective and transformative iconography invoked through funerary equipment.

As previously mentioned, funerary masks and coffins frequently employ golden skin as a means of literally depicting the dead as a divine figure. For instance, the funerary mask of Meret-it-es has little indication of any markers of her personal identity or status in life, but her representation as a god is explicit (*Figure 6*). The skin of the mask is gilded, and her hair is painted blue, another indication of divinity based on the mythology the hair of the gods was made of lapis lazuli, a precious blue stone popular among the Egyptian elite.[19] She dons a tripartite wig, but beyond this there are no other indicators of who Meret-it-es was in life. The only explicit feature of her mask is the assertion of herself as a divine entity.

Similar conclusions can also be drawn from the funerary mask of Tjuiu, again whose most prominent symbolism is the golden skin and blue hair that asserts her divinity (*Figure 7*). Although each mask was able to be assigned to a female individual, there are not any features of the masks alone that explicitly point to this conclusion, asserting that divine transformation took precedent above any other aspects in the masks' design. This trend is generally consistent across funerary equipment and emphasizes how a gendered identity fell secondary to the transformative, funerary process.

Figure 7: The guilded cartonnage mask of Tjuiu. Sourced from Wikimedia Commons. This image is licensed under CC BY-SA 4.0.

Coffins followed similar trends as masks in the display of the deceased individual as gods. Similar conventions of divine skin and hair were obviously

18 Riggs, "Facing the Dead," 96.
19 Goldstein, "Egyptian and Near Eastern Art," 14.

Figure 8: *Outer Coffin of the Singer of Amun-Re, Henettaway.* Metropolitan Museum of Art. This image is in the public domain.

used, but given the depiction of the entirety of the body, the anthropoid coffin had much more space available to assert the same universal concept. The physical features of the coffin presented the dead as a god, but divinity could also be expressed in the artistic framing of the body's position, with "artisans [depicting] Osiris's consorts and protectors Isis and Nephthys at the foot and head of the coffin—as if the dead person inside were the god Osiris himself."[20] Returning to the coffin of Henettawy, this exact scene is present at the foot of her coffin: the two goddesses depicted in mourning.[21] This imagery confirms her divine status through additional conventions beyond her physical features (*Figure 8*). While gender is not necessarily void in any of these pieces of funerary equipment, it is clear that the female individuals they represented were more concerned in asserting their existence as a divine figure over a female identity.

Beyond the equating of oneself to the divine, funerary equipment would invoke transformative and protective symbolism to provide both male and female deceased with the ability to transition to the afterlife. The Egyptian myths surrounding transition into the afterlife heavily employed gendered aspects through an emphasis on the physical act of copulation as the required means of being reborn.[22] Creation myths dictate masculine sperm, rather than the feminine egg, was defined as the source

20 Cooney, "Gender Transformation in Death," 228.
21 Gallery label, "Outer Coffin of the Singer of Amun-Re, Henettwy," Metropolitan Museum of Art, New York, NY.
22 Cooney, "Gender Transformation in Death," 227.

of life, thus centering male ejaculation as a key element of rebirth.[23] It is this symbolic process that required malleable gendered representation to be used; both the male and female deceased required transformation into the literal forms of masculine deities, "and this sacred transformation was thought to grant the dead the ability to create their own rebirth from death."[24] This idea makes clear the necessity of the coffin and mask in this process as it provided the necessary vehicles for this transformation to occur.

Considering this principle, it is clear why these items often lacked gendered symbolism tied explicitly to the identity of the individual they represented. This is especially prevalent in female funerary equipment specifically since women needed to embody the masculine powers in order to achieve a successful transfiguration. This resulted in equipment that embraced an "androgynous but still human-shaped mummy case that provided the dead female with a new and fully bound Osirian body for her transformation."[25] However, this is not to say that women portrayed themselves as men nor that the male deceased did not need to invoke these transformative properties. Rather, both men and women needed to deploy a blend of gendered symbolism, the essential goal of which was transfiguring them into the form of gods.

Figure 9: *Inner Coffin of Amenemopet.* Metropolitan Museum of Art. This image is in the public domain.

These principles are apparent in

23 *Ibid.*, 224.
24 *Ibid.*, 227.
25 *Ibid.*, 228.

the comparison of a female and male coffin. The first coffin, belonging to Amenemopet, a priest of Amun in the Third Intermediate Period, presents clear imagery that transforms him into Osiris (*Figure 9*). The coffin stands with crossed arms, and he is adorned with a false beard, clear markers of Osirian imagery.[26] He also displays the solarizing golden skin and striped lappet wig of blue and gold, indicating his god-like status. Scenes of the gods cover the lower half of the coffin, featuring masculine deities such as Horus, Osiris, and Khepri. Goddesses Isis and Nephthys are also visible flanking the sides of the coffin, and the wings of the sky goddess Nut appear to extend over the coffin's center.

The presence of these goddesses not only invokes protection of the dead,[27] but Nut, who makes up the sky and gives birth to the sun each morning, specifically signifies the coffin as a "microcosm," or a smaller vehicle representative of the universal mythos of death, rebirth, and transformation.[28] While masculine regeneration is necessary for the act of rebirth, the power of feminine protection is equally necessary for completing the process of ascension safely and successfully. It is

Figure 10: *Coffin lid for a mummy. Egypt, ca. 1000 BCE.* Carved and painted wood, inscribed for Henet-Mer, 72 1/2" l. Collection of The Newark Museum. Inv.: 65.65. Art Resource, NY.

26 *Ibid.*, 232:234.
27 *Ibid.*, 228.
28 Ikram and Dodson, The Mummy in Ancient Egypt, 197.

in these depictions that feminine powers are evoked alongside the masculine, "[conveying] and [protecting] the deceased as the body of the sky-goddess Nut, the womb, the egg, and the duat."[29,30]

In analysis of the female coffin of Henet-Mer, all of these principles are invoked almost identically (*Figure 10*). Although she does not have a false beard, her crossed arm pose is a clear display of Osirian imagery in conjunction with her divinizing features. Her figure is androgynous, with no visible molding of breasts, thus avoiding any overt presentations of the female body[31] that may detract from the masculine form she was attempting to embody. The divine scenes depicted on her coffin, while personalized, are reflective of many of the same images present on Amenemopet's. Osiris and Khepri are present, but the viewer's eye is immediately drawn to the large image of Nut in the center, extending her wings across the entirety of the coffin's width. Henet-Mer is clearly depicted in a masculinizing divine form, but feminine power remains a central feature of the coffin. Through the comparison of these two coffins, similarity is much more prevalent than difference, showing an equal implementation of feminine and masculine attributes that would have been necessary for the divine transformation of both the deceased male and female.

In the analysis of the physical artifacts, the complexity of gender and its role in the funerary process becomes apparent. It is clear that the gender identity of the deceased was not a vital element of representation in the coffin and funerary masks, yet these artifacts were not void of gender. The gendered symbolism employed in the design of these pieces of funerary equipment was not primarily intended to represent who the deceased was in life, but rather to aid them in the transformation process that required their divine transfiguration in order to be properly reborn. In order to complete this process successfully, the male and female deceased required a blended invocation of protective feminine and regenerative masculine divine powers provided by the funerary equipment that "represented the container and the contained, the transformation and the outcome."[32]

Inscribing Gendered Meaning: The Role of Funerary Texts

Although it is not the central focus of this exploration, it is important to

29 Cooney, "Gender Transformation in Death," 233.
30 Duat is the realm of the dead in ancient Egyptian mythology.
31 Cooney, "Gender Transformation in Death," 228.
32 *Ibid*., 234.

acknowledge the role of funerary texts in this transformational process. Texts were often written onto coffins directly or placed nearby the dead, and they reflected the necessity of female and masculine elements to evolve for the afterlife. The purpose of texts varied, some offering instruction directly to the deceased, while others invoked the spells necessary for transitioning. Regardless of their exact intent, "mummification and rebirth into the afterlife took the deceased through a process that was governed by the gendered myth of Osiris and ensured by the funerary texts."[33] The heavily gendered aspect of funerary texts is illustrated clearly in a portion of the coffin texts:

> "Isis awakes pregnant with the seed of her brother Osiris. The woman gets up in a hurry, her heart joyful over the seed of her brother Osiris. She says, 'O you gods, I am Isis, Osiris's sister who wept for the father of the gods, Osiris, who settled the massacring of the Two Lands. His seed is within my womb."[34]

In the retelling of the Osirian myth within this text, the masculine and feminine roles fulfilled by the coffin are emphasized in another format. The masculine seed of Osiris acts as the active creation of life, while Isis, the feminine, serves as its protective element. The feminine protection offered by her womb is just as vital as the active seed, and the goddess herself asserts this, declaring "I am Isis, more potent and august than the gods. A god is within my womb, who is the seed of Osiris."[35] This is indicative of the same gendered powers present in the imagery of the coffin and is enforced yet again in texts that would have been written directly upon it, literally inscribing the coffin with another layer of blended gendered meaning.

The transformation of the dead into the masculine form of a god themselves is also validated in the context of funerary texts. Chapter 125 of the Book of the Dead[36] provides complete instructions on how the deceased should confess in the weighing of their soul in the presence of Osiris. The deceased offers praises to the gods, stating "Hail to you gods who are in this Hall of the Two Truths, who have no lies in their bodies, who live on Truth in Heliopolis, who swallow their purification in the presence of Horus who is in his Aten-disk."[37] In a following portion of the chapter, the individual converses with Thoth—the ancient Egyptian god of the moon—prior to their

33 Emily Cole, "The Gendered Individual in Funerary Papyri of the Ptolemaic and Roman Period," J*ournal of the American Research Center in Egypt* 49 (2013): 210. JSTOR.

34 William K. Simpson, "Selections from the Coffin Texts," in *The Literature of Ancient Egypt,* trans. by Robert K. Ritner, William Kelly Simpson, Vincent A. Tobin, and Edward F. Wente, Jr. (New Haven and London: Yale University Press, 2003), 263. Internet Archive.

35 *Ibid.*, 265.

36 The Book of the Dead is a collection of texts written by ancient Egyptian priests from c. 1550 BCE to c. 50 BCE. The book contained spells and instructions meant to assist the deceased through the duat.

37 *Ibid.*, 273.

announcement into the Hall of Two Truths:

> ""To whom, then, shall I announce you?'
>
> 'To him whose roof is on fire, with its walls of living uraei, and the floor of whose house is in flood.'
>
> 'Who is he?'
>
> 'He is Osiris.'
>
> 'Proceed, then. Behold, you are announced. Your bread is the Eye of Horus; your beer is the Eye of Horus, your invocation offerings on earth are the Eye of Horus,' so says he regarding me."[38]

This passage characterizes the deceased and their offerings as Horus and his protections, equating the individual with the same deity established as a revered member of the Egyptian pantheon. With these passages in mind, the transformative property of funerary equipment, regardless of gender, is asserted even more explicitly. Funerary texts were just another means of ensuring the "form which comes into existence after proper rites of mummification have been performed . . . the transfigured mode of being which the deceased acquires through the efficacy of those rites, in which he or she is supposed to endure for the rest of eternity.'"[39] Regardless of the medium, the necessity of the female and male powers were called upon throughout multiple aspects of the transformational process.

Conclusion

Although funerary equipment was in part a means of remembering the deceased, its primary function took on a much more active role in preparing the dead for the afterlife. In the analysis of funerary masks and coffins, it becomes clear that the gendered identity of the individual was secondary to the transformative powers needed to successfully transition to the afterlife. Funerary masks and coffins provided the deceased, both male and female, the means to invoke the divine powers and protections needed to be reborn. These elements of divine iconography incorporated masculine and feminine elements into their design, preventing funerary equipment from being highly evocative of gendered principles as an attempt to evoke the religious properties of both genders in order to transition successfully.

38 *Ibid*., 276–77.

39 Smith quoted in Cole, "The Gendered Individual in Funerary Papyri," 208.

Bibliography

Baines, John and Jaromir Malek. *Cultural Atlas of Ancient Egypt.* New York: Checkmark Books, 2000.

Cole, Emily. "The Gendered Individual in Funerary Papyri of the Ptolemaic and Roman Periods." J*ournal of the American Research Center in Egypt* 49 (2013): 205–18. JSTOR, http://www.jstor.org/stable/24555428.

Cooney, Kathlyn M. "Changing Burial Practices at the End of the New Kingdom: Defensive Adaptations in Tomb Commissions, Coffin Commissions, Coffin Decoration, and Mummification." *Journal of the American Research Center in Egypt* 47 (2011): 3–44. JSTOR, https://www.jstor.org/stable/24555384.

———. "Gender Transformation in Death: A Case Study of Coffins from Ramesside Period Egypt." *Near Eastern Archaeology* 73, no. 4 (December 2010): 224–37. JSTOR, https://www.jstor.org/stable/41103940.

Goldstein, Sidney M. "Egyptian and Near Eastern Art." *Bulletin (St. Louis Art Museum)* 19, no. 4 (1990): 1–52. JSTOR, https://www.jstor.org/stable/40716253.

Ikram, Salima, and Aidan Dodson. *The Mummy in Ancient Egypt: Equipping the Dead for Eternity.* London: Thames and Hudson, 1998.

Reeves, Nicholas. "Amenhotep, Overseer of Builders of Amun: An Eighteenth-Dynasty Burial Reassembled." *Metropolitan Museum Journal* 48, no. 1 (2013): 7–36. JSTOR, https://www.jstor.org/stable/10.1086/675311.

Riggs, Christina. "Facing the Dead: Recent Research on the Funerary Art of Ptolemaic and Roman Egypt." *American Journal of Archaeology* 106, no. 1 (2002): 85–101. JSTOR, https://www.jstor.org/stable/507190.

———. "Roman Period Mummy Masks from Deir el-Bahri." *The Journal of Egyptian Archaeology* 86 (2000): 121–44. JSTOR, https://www.jstor.org/stable/3822312.

Simpson, William K. "Book of the Dead 125: 'The Negative Confession,'" in

The Literature of Ancient Egypt, trans. by Robert K. Ritner, William Kelly Simpson, Vincent A. Tobin, and Edward F. Wente, Jr. 267. New Haven and London: Yale University Press, 2003. Internet Archive, https://ia802907.us.archive.org/1/items/TheLiteratureOfAncientEgyptKellySimpsonBySamySalah/The%20Literature%20of%20Ancient%20Egypt%20-%20Kelly%20Simpson%20By%20Samy%20Salah.pdf.

———. "Selections from the Coffin Texts," in *The Literature of Ancient Egypt*, trans. by Robert K. Ritner, William Kelly Simpson, Vincent A. Tobin, and Edward F. Wente, Jr. 263–66. New Haven and London: Yale University Press, 2003. Internet Archive, https://ia802907.us.archive.org/1/items/TheLiteratureOfAncientEgyptKellySimpsonBySamySalah/The%20Literature%20of%20Ancient%20Egypt%20-%20Kelly%20Simpson%20By%20Samy%20Salah.pdf.

Figure Credits

Figure 1: Mummy of Khnumhotep with mask and broad collar. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/544322.

Figure 2: Funerary Mask of the Overseer of Builders Amenhotep. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/553763.

Figure 3: Funerary Mask of a Woman. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/544824.

Figure 4: Inner Coffin of the Singer of Amun-Re, Henettaway. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/548265.

Figure 5: Inner Coffin of the Singer of Amun-Re, Henettaway. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/548265.

Figure 6: Mummy Mask of Meret-it-es. Egyptian, Late Period to Ptolemaic Period, 30th Dynasty to Early Ptolemaic Dynasty, ca. 380-250 B.C.E. Cartonnage (linen or papyrus covered in plaster), paint, and gold leaf, 15

1/2 x 9 1/2 x 10 inches (39.37 x 24.13 x 25.4 cm). The Nelson-Atkins Museum of Art, Kansas City, Missouri. Purchase: William Rockhill Nelson Trust (by exchange), 2007.12.3. Photo courtesy Nelson-Atkins Media Services. https://art.nelson-atkins.org/objects/53673/mummy-mask-of-meretites.

Figure 7: The guilded cartonnage mask of Tjuiu. Sourced from Wikimedia Commons.This image is licensed under CC BY-SA 4.0. https://commons.wikimedia.org/wiki/File:Egyptian_Museum_(337).jpg.

Figure 8: Outer Coffin of the Singer of Amun-Re, Henettaway. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/548264.

Figure 9: Inner Coffin of Amenemopet. Metropolitan Museum of Art. This image is in the public domain. https://www.metmuseum.org/art/collection/search/544723.

Figure 10: Coffin lid for a mummy. Egypt, ca. 1000 BCE. Carved and painted wood, inscribed for Henet-Mer, 72 1/2" l. Collection of The Newark Museum. Inv.: 65.65. Art Resource, NY. https://gallery.newarkmuseum.org/view/objects/asitem/items$0040:2453504.

A Queer History of Queer Historians

Casen Zelch

Abstract

LGBTQ+ (lesbian, gay, bisexual, transgender, queer, and others) history is a continuously growing area of research and study proven to be important in adding new layers and closing gaps in the history of the United States. However, early queer historians had to lay the groundwork of the discipline while facing prejudice, marginalization, and discrimination from the Lavender Scare of the 1950s to the turn of the century. This text seeks to shed light on the discipline of queer historical research and provide an overview of important early historians and scholars who led to its legitimation today. This text also examines the events and culture of the United States that affected queer scholars and their work. Using diary entries, interviews, memoirs, introductions, and other texts, this paper shows the evolution of queer history as a whole, in itself, and among queer historians during the United States in the mid-to-late twentieth century.

Introduction

Queer history is a relatively new field of study in the United States. The continuously growing area began around the mid-to-late twentieth century, and it was the dedicated work of early queer historians that solidified its place in academia. Although the word "queer" has its own history as a slur, some historians have opted to reclaim the word and use it as an umbrella or simplified term, and this paper does as well. Queer history itself is important, and so are the historians and scholars who study it. This paper considers a "historian" anyone who critically studies and analyzes history because the studying of queer history has inhibited some from the label "academic" or "professional historian" during their lifetime. Knowing who these people are and their place within America's history of homophobia is crucial in understanding their influence. They are an inspiration for aspiring queer scholars and other queer people who want to better understand their history and to draw on past experiences to help ensure a progressing community. Queer history became a legitimate area of academic study today because of early queer historians in the twentieth century who paved the way despite little support, a lack of prior scholarship, and overall queerphobia.

Background on LGBTQ+ People in the Twentieth Century United States

Society perceived homosexuality as a perversion or deviance in the twentieth century United States, which pushed early queer historians to research and write against those presumptions. During the 1950s and 1960s, the U.S. government was compelled to purge themselves of homosexuals, resulting in the Lavender Scare, a "conflation of communism with homosexuality," according to Michael Bronski, the author of *A Queer History of the United States.*[1] Parallel with McCarthy's Second Red Scare with communists, the United States targeted queer people to ensure they remain outside of the government. The Department of State dismissed approximately 1,000 employees over two decades,[2] fearing they were homosexuals and therefore a threat to America. The Lavender Scare produced fear among people that anyone might be a homosexual traitor and bred a fear of exposure and punishment among queer people. In a 1981 diary entry reflecting the culture of the 1950s, gay historian Martin Duberman described the idea of the "homosexual condition" as the inability to commit to another person, and the phenomenon

1 Michael Bronski, *A Queer History of the United States* (Boston: Beacon Press, 2011), 180.
2 *Ibid.*

equaled homosexuality with promiscuity and irresponsibility.[3] Coupling this with the Lavender Scare, the country perceived homosexuals as inherently untrustworthy, and thus they were considered national security risks. Negative attitudes also made it difficult for historians wanting to study non-heterosexuality in the following decades. A prominent queer historian and pioneer of queer studies, John D'Emilio, detailed his personal struggle living in this hostile culture. Religious figures in his life believed trauma was the root of his homosexuality—another stereotype still believed today—but that he could overcome his queerness through self-discipline and prayer.[4] Religion in the United States greatly influenced the perception of perversion, which makes many queer historians like D'Emilio struggle with themselves long before they struggle with careers in queer history. Ideas and assumptions about perversion later gravitated into politics. Political events in the 1970s, such as the "Save Our Children" campaign in Florida or California's Proposition 6 (also known as The Briggs Initiative), were prominent parts of the anti-gay politics. The Florida campaign sought to reverse the ban on sexual orientation discrimination on the basis of children's' perceived need to understand Biblical morality.[5] Additionally, Senator John Briggs' Proposition 6 in California was an attempt to ban homosexuals and allies from teaching in public schools, stating, "We've got to be crazy to allow homosexuals who have an affinity for young boys to teach our children."[6] While these campaigns aimed to push anti-gay stereotypes, such as immorality and pedophilia, to limit career success for homosexuals, it also inspired historian Allan Bérubé to begin to research the history of gay oppression.[7] It was from this intense anti-queerness in the twentieth century that led to a growth of people, such as Bérubé and D'Emilio, to start investing in queer history to combat the negative notions surrounding homosexuality. Unfortunately, however, the 1980s and early 1990s was an era of illness, fear, and mortality for the LGBTQ+[8] community, affecting queer people socially and politically in the United States.

The AIDS crisis devastated the LGBTQ+ community, specifically gay men, and pushed some to better document and record queer people and history. AIDS, coined the "Gay Plague," took a toll on many facets of soci-

3 Martin B. Duberman, "Gay In The Fifties," *Salmagundi*, no. 58/59 (1982): 48. JSTOR.

4 John D'Emilio, introduction to *Making Trouble: Essays on Gay History, Politics, and the University* (United Kingdom, Europe: Routledge, 2014), xvii. Internet Archive.

5 John Gallagher and Chris Bull, P*erfect Enemies: The Religious Right, the Gay Movement, and the Politics of the 1990s* (New York: Crown Publishers, 1996), 16–17.

6 Penelope McMillan, "Briggs Points to Gay Teacher in North as Example," *Los Angeles Times*, September 29, 1978.

7 John D'Emilio and Estelle B. Freedman, introduction to M*y Desire for History: Essays in Gay Community, and Labor History,* by Allan Bérubé (Chapel Hill: University of North Carolina Press, 2011), 8. eBook Community College Collection (EBSCOhost).

8 Lesbian, gay, bisexual, transgender, queer, and others.

ety, including doctors, researchers, public health officials, and the LGBTQ+ community.[9] The epidemic affected men who had sex with men the most because they were more susceptible in contacting HIV at the time.[10] The epidemic took 12,000 Americans by 1986, and rather than having interest in preventing the disease, the government seemed preoccupied only with testing them.[11] Against the backdrop of a rising death toll, many queer people who survived the AIDS epidemic were ultimately exposed and forced out of the closet, either from being outed from testing or for knowing others who died from the disease. This exposure resulted in the creation of large social networks and organizations, especially for intellectuals and queer historians.[12] Bérubé—through these dense networks of queer writers, scholars, and intellectuals—found opportunities to teach about his work in the 1990s such as his research on gay GIs in World War II (WWII).[13] Regardless of whether the government or the general public would accept queer people and queer work, queer historians found a new network to start discussing and developing their scholarship.

The Gay Liberation Movement and Queer Culture

The rise of gay culture and the gay liberation movement showed the need for documentation for this historical time. While the outing of queer people was an "attempt to punish, 'manage,' or isolate gay Americans," it also solidified a queer identity and growing minority.[14] It appeared that no matter how hard society attempted to silence this community, they nevertheless persisted. Major cities, known for being more socially progressive than rural towns, served as a haven for queer people to move and live. In New York City, the gay liberation movement of the 1970s allowed queer people to reject predetermined, conventional ideas surrounding sexuality and expression such as being in heterosexual relationships or keeping their sex lives private. From the 1960s to the 1980s, the sexual revolution allowed queer people to experiment with each other, often at gay bars or bathhouses.[15] Big cities provided numerous places for fellow queer people to meet and bond with others, which resulted in a growing, tight-knit community that soon found itself to be a powerful force. A significant amount of known LGBTQ+ history took place

9 Allan Bérubé, *My Desire for History: Essays in Gay Community, and Labor History*, (Chapel Hill: University of North Carolina Press, 2011), 62. eBook Community College Collection.
10 Eric Cervini, *The Deviant's War: The Homosexual vs. United States of America* (New York: Farrar, Straus, and Giroux, 2020), 376.
11 *Ibid.*
12 D'Emilio and Freedman, introduction to *My Desire for History,* 24.
13 *Ibid.*, 24:27.
14 Bérubé, *My Desire for History*, 111.
15 Alex Espinoza, *Cruising: An Intimate History of a Radical Pastime* (Los Angeles: The Unnamed Press, 2019), 96.

in these big cities, most notably the Stonewall Riots, which was important for queer historians to document. Queer organizations and secret societies, namely the Mattachine Society and the Daughters of Bilitis, formed in New York City and Los Angeles.[16] The purposes of these organizations were to unify homosexuals to research, educate their community, and create a collective culture to in turn produce social and political power. D'Emilio argues the process of creating a community has often involved "the search for a usable past," as shown by Black Americans in the Civil Rights Movement as well as women in the feminist movement.[17] These movements used symbolic protest, such as silence and invisibility, to highlight their oppression, which also inspired D'Emilio to find this community and make their efforts visible.[18] However, he stresses the difficulty in simply recounting the early stages of the movement was because of its complexity. The gay liberation movement "became a story of consciousness as well, a story of creation, of the making of a minority,"[19] so D'Emilio and other queer historians found a need to document the historic time they themselves were living through. Political events such as the Lavender Scare and the AIDS crisis demonstrated how easily the public could remove queer people from public life and history, so there was an added weight to ensure queer history would remain. Although anti-queer settings in the United States were disadvantageous for queer people, this atmosphere also fueled desires to grow a foundation of community and knowledge that assisted queer historians in gaining acceptance in academia.

Discrimination in Academia

The "Save Our Children" campaign and the Briggs Initiative highlight two strides the country took to force queer people out of their careers in public education. These are just two events in the decades-long attempt to block queer academics from employment and research in queer studies. In 1948, the University of Missouri accused Professor E.K. Johnson of sodomy, and he was fired.[20] Whether or not Professor Johnson showed any interest in queer knowledge, the mere accusation of homosexuality was reason enough for many academics during the time to be reluctant about coming out. A 1982 Chronicle story reported queer college faculty and administrators feared losing their careers, and they were reluctant to "come out" on campuses.[21]

16 Cervini, *The Deviant's War*, 69: 115–16.

17 D'Emilio and Freedman, introduction to *My Desire for History,* 8.

18 John D'Emilio, *In a New Century: Essays on Queer History, Politics, and Community Life* (Madison: University of Wisconsin Press, 2014), 90.

19 *Ibid.*

20 "Lillian Faderman Tells the Gay Story to Date," Interview by Mary Meriam, *The Gay and Lesbian Review Worldwide*, 22. no. 5 (2015): 23. Biography Reference Bank.

21 Lawrence Biemiller, "Homosexual Academics Say 'Coming Out' Could Jeopardize Careers," in "The Godfather

The same year, an American Sociology Association task group documented patterns of discrimination and homophobic attitudes that university sociology department heads possess, and how those attitudes may have inhibited gay scholarship. Researches concluded being "out" was a risk for gay people wanting careers, which further inhibited pursuit of queer topics.[22] D'Emilio discussed the task force's findings as it relates to queer historians and his own personal experiences of discrimination. There is a great significance in these prejudicial and homophobic attitudes when it comes to him and his students studying history, he said, such has needing to rephrase gay history to "history of sexuality" to get research approved or having only two students interested in queer history.[23] D'Emilio did have professional success in obtaining research grants, but his successes came only after making his work more palatable. The United States government, however, dismissed non-heterosexual history altogether and controlled history itself by purposely hiding or ridding mass amounts of civilian and military records from the public view. Bérubé's most notable research, *Coming Out Under Fire: The History of Gay Men and Women in World War Two*, highlighted a gay and lesbian GI cooperative, but many of the documents that made up the foundation of Bérubé's research had not previously come to light because authorities had regularly withheld government, civilian, and military records on the subject of homosexuality.[24] The deep prejudice within the U.S. government and schools made it exceptionally difficult for queer academics' employment and their research interests in queer studies.

Many universities and publications refused to support queer theory and research. Bérubé had to become a self-trained historian, writing and researching without the support the academy would provide to professors.[25] Although he could not obtain the monetary and material benefits a university could supply, this allowed Bérubé to be more of a community-based historian, which became one of the defining characteristics of his career. Community-based historians focused on the untold histories excluded from traditional history, focusing more on public histories surrounding everyday people such as oral history. D'Emilio praised the impact community-based historians had on queer history. He considered their projects equaled to professional research.

---

of Gay Studies," *Chronicle of Higher Education* 25, no. 8 (2018): 9.

22 John Gagnon et al., "Report of the American Sociological Association's Task Group on Homosexuality," *American Sociologist* 17, no. 3 (August 1982): 164. SocINDEX with Full Text.

23 John D'Emilio, "Not a Simple Matter: Gay History and Gay Historians," *Journal of American History* 76, no. 2 (1989): 442:437. JSTOR.

24 Clayton Koppes, review of "Coming Out Under Fire: The History of Gay Men and Women in World War Two (Book)," *Journal of American History* 78, no. 1 (June 1991): 377–78.

25 John D'Emilio and Estelle B. Freedman, foreword to *Coming Out Under Fire: The History of Gay Men and Women in World War Two*, by Allan Bérubé, (Chapel Hill: University of North Carolina Press, 2010), viii.

Plus, the research was more accessible to everyday audiences, creating interest and commitment to their work.[26] Some queer historians found success through these community-based projects, all while professional queer historians still struggled for approval of their research. Historian Martin Duberman said he wanted to research and teach sexual behavior and the history of lesbian and gay people, and he told his university so in 1977. His institution's response was "undiluted horror," and the course was rejected on the grounds that sexual history was not a formal area of study.[27] Lawrence Biemiller further accounts for this prejudice in professional settings when he said his colleges refused to fund research on the subject of homosexuality even after academics had published papers about it.[28] This attitude universities presented with Duberman and Biemiller were not at all uncommon during the twentieth century, and the pushback these scholars accounted show how discrimination hindered queer scholarship. In turn, a lack of encouragement from scholarship as a whole isolated queer historians from the support of institutions and also from colleges in the history field interested in queer studies, which lead to "material as well as to cultural impoverishment and decline."[29] Universities and other institutions inhibited the growth of queer studies, affecting both queer history and historians, and only a few received proper acknowledgment. While community-based historians had more positive community engagement, they lacked the material and sometimes intellectual support from other historians. Professional historians, on the other hand, had to bounce around their topics, sometimes replacing their interests with more acceptable ones, in order to receive monetary and material benefits.

Queer Historians' Fight for Validity

To prove queer history was a valid and essential area of study, early queer historians had to use what little prior scholarship was available to them. Compared to mainstream historical topics, such as military or presidential history, challenges that came with researching queer history included a lack of literature, definitions, and other sources.[30] Early queer historians had to lay the first groundwork of information, while other historians at the time had a vast amount of knowledge to help with their research. However, this did not stop queer historians from using previous scholarship and creating more of it. Given the United States' history and culture around sexuality is incred-

26 D'Emilio, "Not a Simple Matter," 438.
27 Martin B. Duberman, "Reclaiming the Gay Past," *Reviews in American History* 16, no. 4 (December 1988): 515.
28 Biemiller, "'Coming Out,'" 10.
29 Lisa Duggan, "The Discipline Problem: Queer Theory Meets Lesbian and Gay History," J*ournal of Lesbian and Gay Studies* 2, no. 3 (June 1995): 179.
30 D'Emilio, "Not a Simple Matter," 437.

ibly conservative, documents with sexual content were regularly censored, unpublished, or simply ignored by other scholars.[31] Queer academics and queer historians at the time were able to phish through previously researched documents and find vital information that could help further queer history. Historian George Chauncey was also able to use oral histories, along with other unpublished documents, for his studying of "the gay world" in New York City before the 1960s.[32] By using oral histories, Chauncey constructed a richer and more personal history, and he subsequently created his own sources to analyze history. All historians, especially queer historians, have found oral histories valuable in shedding light on the personal biographies and perspectives of certain eras that other major mainstream history fields often overlook.

Areas of history with ample research and publications, such as presidential and military histories, served as avenues for early queer historians to show the validity of queer history, examining it through both general and interdisciplinary lenses. Hoping to get his book *Coming Out Under Fire* published, Bérubé merged the topic of gay and lesbian soldiers in WWII to a broader social and political history of the government during the time.[33] With his research, he added a new perspective about the war, conveying the importance of gays and lesbians in U.S. history, and how their participation simultaneously conflicted with the anti-gay policies the government and military implemented.[34] Bérubé successfully included queer people and their impact on mainstream history, all while emphasizing the need for additional queer history. He continued this interconnectedness of sexuality and power when writing about queer people in labor history, arguing his research would better help understand the effects sexuality can have at work. He included the term "heterosexualization"[35] to describe how heterosexuality systemically affects everyone in their work environments. By connecting his niche research to another general areas such as labor history, Bérubé continued establishing the validity of queer history as any other broad area of study. Duberman also connected its significance by affirming that queer history is key to queer people understanding their past, and that the discipline also aims to tell about all human beings and human nature.[36] Both Duberman and Bérubé present queer history as a standalone area that compliments other history fields. By

31 John D'Emilio and Estelle B. Freedman, *Intimate Matters: A History of Sexuality in America* (Chicago: University of Chicago Press, 2012), xiv.

32 George Chauncey, *Gay New York: Gender, Urban Culture, and the Making of the Gay Male World* (New York: Basic Books, 1994), 370.

33 D'Emilio and Freedman, foreword to *Coming Out Under Fire*, by Allan Bérubé, viii.

34 D'Emilio and Freedman, introduction to *My Desire for History*, 17–18.

35 Bérubé, *My Desire for History,* 251.

36 Duberman, "Reclaiming the Gay Past," 523–24.

treading into other areas, queer history has at some points, "remapped" other fields of history, for instance narrowing the broad U.S. military and political history of WWII to power dynamics between the government and its queer citizens.[37] Queer historians showed the power both they and their research had in questioning and unsettling prior understanding of history. They truly dedicated themselves to ensure queer history could stand on its own with other fields of history and as an inclusion to history itself.

Even after the crucial work queer historians made, they were subject to criticism from both outside and within their community. In 1975, women's and gender historian Carroll Smith-Rosenberg warned of the potential problems historians face when defining and analyzing same-sex relationships purely through a psychological framework and not also a cultural and social one. She argued psychological theories surrounding sexuality at the time dealt with their own criticisms of a normal-abnormal dichotomy and origins of "deviance," and she questioned if there was any historical evidence historians could use to support these theories.[38] Her critiques helped early queer historians question their approaches and brought more conservations. In 1990, after a few decades of built-up queer scholarship, D'Emilio and Estelle Freedman, who specializes in women's and feminist studies, pointed to a new problem in writing the history of sexuality. For them, the prior lack of sources was no longer a problem, but rather a lack of clear definitions of subjects surrounding the history of sexuality, particularly in relation to gender and class.[39] They acknowledged the progress research from this field made but stressed the need for improved cultural and social frameworks much like Smith-Rosenberg did. Without clear definitions, D'Emilio and Freedman claimed queer history faces other issues.

Queer academics also faced the issue of whether they could support histories and biographies that assumed a person was "gay" in a modern sense without substantial historical evidence to support the assumption.[40] For example, it is incorrect to label Achilles and Patroclus as gay lovers because Ancient Greece's social and cultural framework would consider them in either a pederastic relationship or loving friendship, which is unlike our modern understanding of queer identities and relationships.[41] Using modern terms and un-

37 Regina Kunzel, "The Power of Queer History." *American Historical Review* 123, no. 5 (December 2018): 1582, Oxford Academic.

38 Carroll Smith-Rosenberg, "The Female World of Love and Ritual: Relations Between Women in Nineteenth-Century America," *Signs* 1, no. 1 (Autumn 1975): 1–2.

39 Estelle B. Freedman and John D'Emilio, "Problems Encountered in Writing the History of Sexuality: Sources, Theory and Interpretation," Journal of Sex Research 27, no. 4 (1990), 481. JSTOR.

40 James V. Carmichael Jr., "'They Sure Got to Prove It on Me': Millennial Thoughts on Gay Archives, Gay Biography, and Gay Library History," *Libraries & Culture: A Journal of Library History* 35, no. 1 (2000): 94. JSTOR.

41 David M. Halperin, "How to Do the History of Male Homosexuality," *GLQ: A Journal of Lesbian & Gay Studies* 6, no.1 (January 2000): 90. Project Muse.

derstanding to determine a historical figure's sexuality without acknowledging their respective sociocultural historical context is problematic when researching queer history. In raising these criticisms, queer historians have sharpened their discipline into a credible area of study. Anthropology professor John Borneman reviewed D'Emilio's book, *Making Trouble: Essays on Gay History, Politics, and the University*, and praised his honesty and somewhat informal writing as "high standard of historiography,"[42] adding to D'Emilio's credibility and substantial career as a queer historian. However, Borneman also criticized younger queer scholars for their adherence to psychoanalytical and literary frameworks and not historical ones. He argued for a merge of "disciplines" when researching queer history, exampling gay author James Baldwin's literary work with D'Emilio's historical analysis in *Making Trouble*.[43] Written in 1994, the criticism ultimately contributes to queer history's validity as a worthy field for academic critique. It also displayed D'Emilio's evolving career as a queer historian as other scholars pointed to him as a model for historical research and academic writing.

Progress Towards the Finish Line

The work of early queer historians slowly but surely gained appreciation in the academic world, proving its legitimacy. Bérubé later received a MacArthur grant, funding that had never before been granted to someone for their queer scholarship.[44] The award allowed him to continue writing about queer history and proved his past and future work were significant contributions to the academic world. For D'Emilio, the University of North Carolina's history department hired and gave him tenure, supporting his work and identity, which he deemed "a gay success story."[45] He originally had concerns about his career when he wanted to pursue queer history, but his dedication to form this new field eventually paid off with more opportunities in the progressing academic world. After 25 years, D'Emilio and Freedman published their book, Intimate Matters: A History of Sexuality in America, the first full-length study of the history of sexuality in the United States. The book details the complexities of sexuality and many factors still prominent in discussions, and it provides a useful point of reference to show how the field of sexuality has grown.[46] Freedman reflected on this progress and noted the gaps

42 John Borneman, review of "Making Trouble: Essays on Gay History, Politics, and the University," by John D'Emilio. *Journal of History of Sexuality* 4, no. 3 (1994): 480. JSTOR.

43 *Ibid.*

44 D'Emilio and Freedman, introduction to *My Desire for History*, 30.

45 D'Emilio, "Not a Simple Matter," 435.

46 Brigitte Fielder, review of "Intimate Matters: and Documenting Intimate Matters: Primary Sources for a History of Sexuality in America Ed. by Thomas A. Foster," *Early American Literature* 50, no. 2 (June 2015): 605, Project Muse.

of knowledge closed with each revision of the book thanks to the growing diversity within the study of sexuality.[47] Like every other field of history, early queer historians showed appreciation for their colleagues' work, but they also acknowledged the work still needed to progress queer history even further, such as analyzing the intersectionality of sexuality with race, class, and other identities throughout American history.

Queer women's research on lesbian existence tended to be left out of queer history since the discipline was dominated by the history of gay men. Historian Lillian Faderman garnered critical praise for her works on lesbian history, most notably her 1981 book, S*urpassing the Love of Men: Romantic Friendships and Love Between Women from the Renaissance to the Present.* Faderman compiled hundreds of literary and historical documents spanning from the sixteenth to the twentieth centuries and analyzed love between women.[48] Focusing on the historical importance of women loving women, Faderman provided additional layers of gender and female sexuality often excluded from history, and her work validated the lesbian existence in queer history. In 1980 Adrienne Rich, a queer scholar and poet, wrote her radical essay, "Compulsory Heterosexuality and Lesbian Existence," in which she argued heterosexuality is a historically male-constructed institution that forces the oppression of women.[49] Rich complicates the basis of heterosexuality as the norm and insisted feminists consider the "lesbian continuum:" the idea that women's emotional and sexual bonding is how women can defeat patriarchal control.[50] Both Faderman and Rich connected their study of lesbian sexuality with feminist and historical scholarship in a way that demanded visibility.

Queer historians' work did not just legitimize queer history, it also allowed queer people outside academia to see into their community's past and empower themselves. Queer people now look to the past to help understand their experiences, the factors that have been inhibiting their behaviors, and how other queer people before dealt with their sexualities throughout their lives.[51] With the work of community-based history projects and published works, early queer historians provided a vast amount of accessible knowledge of the past for historians and everyday people to find. The 1979 San Francisco Lesbian and Gay History Project, for example, believed shared queer history would empower the community to act more effectively, both socially and

47 Estelle B. Freedman, "Comment on 'Intimate Matters After Twenty-Five Years,'" *Frontiers: A Journal of Women Studies* 35, no. 1 (2014): 42, JSTOR.

48 Lillian Faderman, *Surpassing the Love of Men: Romantic Friendship and Love Between Women from the Renaissance to the Present* (New York: William Morrow, 1981).

49 Adrienne Rich, "Compulsory Heterosexuality and Lesbian Existence," *Signs* 5, no. 4 (July 1980): 633, JSTOR.

50 *Ibid.*, 648–59.

51 Duberman, "Reclaiming the Gay Past," 523.

politically.[52] Additionally, queer history and what it has produced cannot be geared towards the intellectuals and scholars exclusively, but rather the whole public because history is integral for queer people and their community.[53] The difficult work queer historians have done and continue to do are crucial for the LGBTQ+ community so future queer historians and queer people can better understand their history and ensure the success of their community continues.

Queer history is now a legitimate area of academic study today, but it is only so thanks to queer historians of the twentieth century. These historians and scholars experienced historical events affecting the LGBTQ+ community and intense queerphobia during the mid-to-late th century, which inspired them to document and pursue queer history. Despite little support and a lack of prior scholarship, they formed a strong network that allowed them to share knowledge and lay a foundation essential for a marginalized community from which to build up. Their dedicated work proved to be rewarding as queer historians later had successful careers: winning awards, obtaining grants, and inspiring younger queer people to pursue the discipline. Queer history flourished into an interdisciplinary field and continues to grow every day, adding more knowledge about the significance of queer people and their community within America's history.

52 D'Emilio and Freedman, introduction to *My Desire for History,* 11.

53 Claude J. Summers, "Gay Voices, Gay Genealogies," *American Literary History* 5, no. 1 (1993): 157, JSTOR.

Bibliography

Bérubé, Allan. *My Desire for History: Essays in Gay, Community, and Labor History*. Chapel Hill: University of North Carolina Press, 2011. eBook Community College Collection (EBSCOhost), https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=e900xww&AN=365994&site=eds-live&scope=site&custid=039-820.

Biemiller, Lawrence. "Homosexual Academics Say 'Coming Out' Could Jeopardize Careers." *Chronicle of Higher Education* 25, no. 8: (2018): 9–10. Quoted in Lawrence Biemiller, "The Godfather of Gay Studies." Chronicle of Higher Education, https://www.chronicle.com/article/the-godfather-of-gay-studies/?bc_nonce=wf0t5t5hm91csyru167l2&cid=reg_wall_signup.

Borneman, John. Review of Making Trouble: Essays on Gay History, Politics, and the University, by John D'Emilio. *Journal of the History of Sexuality* 4, no. 3, (1994): 478–80. JSTOR, https://www.jstor.org/stable/3704366.

Bronski, Michael. *A Queer History of the United States.* Boston: Beacon Press, 2011.

Carmichael, James V., Jr. "'They Sure Got to Prove It on Me': Millennial Thoughts on Gay Archives, Gay Biography, and Gay Library History." *Libraries & Culture: A Journal of Library History* 35, no. 1 (2000): 88–102. JSTOR, https://www.jstor.org/stable/25548801.

Cervini, Eric. *The Deviant's War: The Homosexual vs. The United States of America*. New York: Farrar, Straus, and Giroux, 2020.

Chauncey, George. *Gay New York: Gender, Urban Culture, and the Making of the Gay Male World, 1890–1940*. New York: Basic Books, 1994.

D'Emilio, John. *In a New Century: Essays on Queer History, Politics, and Community Life*. Madison: University of Wisconsin Press, 2014. eBook Collection (EBSCOhost), https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=nlebk&AN=777473&site=eds-live&scope=site&custid=039-820.

———. *Making Trouble: Essays on Gay History, Politics, and the University.*

United Kingdom, Europe: Routledge, 2014. Internet Archive, https://archive.org/details/makingtroubleess00demi.

———. "Not a Simple Matter: Gay History and Gay Historians." *The Journal of American History* 76, no. 2 (1989): 435–42. JSTOR, https://www.jstor.org/stable/1907977.

D'Emilio, John, and Estelle B. Freedman. Foreword to *Coming Out Under Fire: The History of Gay Men and Women in World War II*. by Allan Bérubé, vii-xiii. 20th anniversary ed., Chapel Hill: University of North Carolina Press, 2010. https://search.ebscohost.com/login.aspx?direct=true&AuthType= so&db=e900xww&AN=343674&site=eds-live&scope=site&custid=039-820.

———. *Intimate Matters: A History of Sexuality in America*. 3rd ed., Chicago: University of Chicago Press, 2012.

———. Introduction to *My Desire for History: Essays in Gay, Community, and Labor History.* by Allan Bérubé, 1–37. University of North Carolina Press, 2011. eBook Community College Collection (EBSCOhost),https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=e900xww&AN=365994&site=eds-live&scope=site&custid=039-820.

Duberman, Martin B. "Gay In The Fifties." *Salmagundi*, no. 58/59 (1982): 42–75. JSTOR, https://www.jstor.org/stable/40547564.

———. "Reclaiming the Gay Past." *Reviews in American History* 16, no. 4 (December 1988): 515–25. JSTOR, https://www.jstor.org/stable/2702347.

Duggan, Lisa, "The Discipline Problem: Queer Theory Meets Lesbian and Gay History," In Journal of Lesbian and Gay Studies 2, no. 3 (1995): 179–191. Duke University Press, https://doi-org.eu1.proxy.openathens.net/10.1215/10642684-2-3-179.

Espinoza, Alex. *Cruising: An Intimate History of a Radical Pastime*. Los Angeles: The Unnamed Press, 2019.

Faderman, Lillian. *Surpassing the Love of Men: Romantic Friendship and Love Between Women from the Renaissance to the Present*. New York: William

Morrow, 1981.

Fielder, Brigitte. Review of "Intimate Matters: A History of Sexuality in America, 3rd Edition by John D'Emilio and Estelle B. Freedman, and: Documenting Intimate Matters: Primary Sources for a History of Sexuality in America Ed. by Thomas A. Foster." *Early American Literature* 50, no. 2 (June 2015): 605–11. Project Muse, https://doi.org/10.1353/eal.2015.0040.

Freedman, Estelle B. "Comment on 'Intimate Matters after Twenty-Five Years.'" *Frontiers: A Journal of Women Studies* 35, no. 1 (2014): 41–42. JSTOR, https://doi.org/10.5250/fronjwomestud.35.1.0041.

Freedman, Estelle B., and John D'Emilio. "Problems Encountered in Writing the History of Sexuality: Sources, Theory and Interpretation." *Journal of Sex Research* 27, no. 4 (1990). 481–95. JSTOR, https://www.jstor.org/stable/3812770.

Gagnon, John, Suzanne Keller, Ronald Lawson, Patricia Miller, William Simon, and Joan Huber. "Report of the American Sociological Association's Task Group on Homosexuality." *American Sociologist* 17, no. 3 (August 1982): 164–80. SocINDEX with Full Text, https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=sih&AN=4944061&site=eds-live&scope=site&custid=039-820.

Gallagher, John, and Chris Bull, *Perfect Enemies: The Religious Right, the Gay Movement, and the Politics of the 1990s*. New York: Crown Publishers, 1996.

Halperin, David M. "How to Do the History of Male Homosexuality." *GLQ: A Journal of Lesbian & Gay Studies* 6, no.1 (January 2000). Project Muse, muse.jhu.edu/article/12121.

Koppes, Clayton R. Review of Coming Out Under Fire: The History of Gay Men and Women in World War Two, by Allan Bérubé. *Journal of American History* 78, no. 1 (June 1991): 377–78. Humanities International Complete, https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=hlh&AN=9107011033&site=eds-live&scope=site&custid=039-820.

Kunzel, Regina. "The Power of Queer History." *American Historical Review* 123, no. 5, (December 2018): 1560–82. Oxford Academic, https://doi.org/10.1093/ahr/rhy202.

"Lillian Faderman Tells the Gay Story to Date," Interview by Mary Meriam. The Gay and Lesbian Review Worldwide, 22. no. 5 (2015): 23. *Biography Reference Bank*, https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=brb&AN=109151308&site=eds-live&scope=site&custid=039-820.

McMillan, Penelope. "Briggs Points to Gay Teacher in North as Example." *Los Angeles Times*, September 29, 1978. https://www.newspapers.com/image/384926584.

Rich, Adrienne. "Compulsory Heterosexuality and Lesbian Existence." *Signs* 5, no. 4 (July 1980): 631–60. JSTOR, https://www.jstor.org/stable/3173834.

Smith-Rosenberg, Carroll. "The Female World of Love and Ritual: Relations between Women in Nineteenth-Century America." *Signs* 1, no. 1 (Autumn 1975): 1–29. JSTOR, https://www.jstor.org/stable/3172964.

Summers, Claude J. "Gay Voices, Gay Genealogies." *American Literary History* 5, no. 1 (1993): 147–58. JSTOR, https://www.jstor.org/stable/489765.

McQueary College of Health and Human Services

Effects of Reproductive Hormones on Ovarian Cancer Development and Potential Treatments to Counteract Malfunctioning Hormonal Signaling Pathways

Emily Chisum, Richard Garrad, PhD, and Molly Greenwade, MD

Abstract

Ovarian cancer remains the most lethal gynecologic cancer and a top cause of death among females in the United States. While the exact cause of the cancer is unknown, there are many proposed mechanisms involving biological, environmental, and genetic factors. Unfortunately, there are currently no screening methods available to detect ovarian cancer, and it is often diagnosed at an advanced stage using an ultrasound technology that identifies tumors. Late diagnosis, in combination with the aggressive nature of ovarian tumors and lack of effective treatment options, has led to poor survival. Recently, scientists have been working to identify potential causes for ovarian cancer and methods for detection. One area they have investigated is reproductive hormones and their influence on ovarian function. Estrogen and progesterone have a host of functions and are essential in regulating signal transduction pathways that are involved with several biologic processes such as cell proliferation, apoptosis, gene expression, and follicular development. When hormone signaling pathways malfunction, women are at a greater risk for ovarian cancer development. A better understanding of hormone signaling pathways and future development of drug therapies that can target faulty hormonal signaling pathways can potentially lead to better outcomes. This research will focus on hormonal pathways and functions and will outline how disruptions in these normal cellular processes may be implicated in ovarian cancer development, progression, and prognosis.

Introduction

Cancer has persistently remained one of the leading causes of death in the United States, and reproductive cancers account for a large majority of female cancers. Specifically, ovarian cancer is the fifth most common cancer death among women in the United States and is the leading cause of gynecological cancer deaths ("Key Statistics for Ovarian Cancer"). In developed countries, the five-year survival rate of ovarian cancer is approximately 47% (Lheureux et al., 2019). This is due in large part to ovarian cancer's delayed diagnosis and advanced stage at presentation, and the majority of diagnoses are at stage III or IV of the disease (Luo et al., 2017). Over the past few decades, research for other cancer types has improved survival rates as a result of better prevention methods, improved screening modalities, and more effective treatment options. However, ovarian cancer research has yet to find an effective screening test to improve survival outcomes. Currently, there are no FDA approved screening tests to detect ovarian cancer, and the warning signs, such as weight loss, bloating, abdominal fullness and pain, and increased abdominal girth, are vague. If detected at an early stage, ovarian cancer survival rates range from 75–95%. When detected at an advanced stage, the treatment usually entails cytoreductive surgery and platinum-based chemotherapy with the goals of removing tumor tissue surgically and killing cancerous cells respectively. Response rates after these initial therapies are high; however, unfortunately, approximately 60–70% of patients will recur within 18 months of treatment. (Luo et al., 2017). Numerous factors can contribute to recurrence: Chemotherapy resistance and not removing all of the tumor tissue initially may cause the cancer to grow back. For these reasons, developing an effective screening test is crucial for early detection and improvement in outcomes. Every cancer is unique in how it is detected, its genetic makeup, symptoms, and malignant potential. There are also a number of influences leading to cancer development, including biological, genetic, and environmental factors. One of the major barriers to improving cancer detection is pinpointing the exact cause of ovarian cancer. Risk factors for ovarian cancer have been well-described, and these include early menarche, late menopause, older age at first pregnancy, and nulliparity. Some potential protective factors against ovarian cancer have also been identified, such as oral contraceptives. These medications reduce the risk of ovarian cancer by decreasing the levels of circulating ovarian estrogen levels and decreasing the number of ovulatory cycles. These findings suggest the number of ovulatory cycles a woman experiences is positively correlated with the incidence of ovarian cancer, and when ovulation frequency is minimized or disrupted, the more protected the ovaries

are from developing cancer. This also suggests hormones may play a role in the development of ovarian carcinoma (Langdon et al., 2020). One of the ways researchers and clinicians try to diagnose cancer is through biomarker detection associated with a particular disease, and researchers have worked to identify a number of biomarkers associated with ovarian cancer. The primary focus of this research is to describe biomarkers associated with hormones and their effect on ovarian cancer development, progression, and prognosis. The beginning of this research will introduce ovarian structure and function, staging and types of ovarian cancer, and signal transduction pathways that play a role in regulating ovarian cancer formation. The second part of this research will discuss hormones associated with ovarian function and regulation, how hormone signaling dysfunction affects ovarian cancer formation, and potential targets for treatment of ovarian cancer related to hormone signaling regulation.

Ovary Structure and Function

The ovary is the female-differentiated gonad composed of two different types of cells: germ cells and somatic cells. Germ cells include the oocytes or eggs that can become fertilized, leading to pregnancy. Somatic cells, on the other hand, can include granulosa cells, thecal cells, and stromal cells. These cells specialize in follicular development, ovulation, and corpus luteum formation, which is a gland formed after ovulation that is required for pregnancy (Richards & Pangas, 2010). Both germ and somatic cell function and regulation relies heavily on endocrine signaling. One of the functions of the ovary, ovulation, is reliant on an endocrine signaling pathway that starts in a structure within the brain called the hypothalamus. The hypothalamus releases a hormone called gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). If GnRH pulses are low in frequency, FSH levels increase, which signifies early stages of the menstrual cycle. However, higher GnRH pulses cause increases in LH levels, leading to the LH surge and causing ovulation and formation of the corpus luteum (Richards & Pangas, 2010). This signaling pathway cycles continually and is impacted by a number of other hormones, such as estrogen and progesterone, which contribute to normal ovarian function. Malfunction of any of these molecules or mechanisms can possibly contribute to formation of ovarian cancer by affecting processes that selectively kill cells, such as apoptosis, those that promote proliferation of cells, or by other signaling pathways indirectly associated with these processes.

Ovarian Cancer Pathology

Stages of Ovarian Cancer

Ovarian cancer progression is defined by four distinct stages. During stage I of the disease, the patient exhibits a tumor that is confined only to the ovaries or fallopian tubes. This stage begins in only one of the ovaries or fallopian tubes without any cancer presence on the surface of these structures and without any cancer spreading to lymph nodes or distant sites. By the end of stage I, the cancer has spread to one or both ovaries or fallopian tubes as well as the surfaces of these structures. Cancer cells also exist in the fluid within the peritoneal region. Stage II begins with tumor presentation inside one or both ovaries or fallopian tubes. The cancer has also extended to the uterus and other pelvic intraperitoneal tissues by the end of this stage. During stage III, the tumor is present in one or both ovaries or fallopian tubes, cancer spread is confirmed cytologically or histologically to the peritoneum outside of the pelvic region. Metastasis might also be observed to retroperitoneal lymph nodes by the end of the stage. Finally, stage IV ovarian cancer occurs when metastasis starts to overtake more distant regions of the body (Lheureux et al., 2019). Cancerous cells can be found in places such as the spleen, liver, lungs, bone, or lymph nodes outside of the retroperitoneal region. It is important to know that ovarian cancer is unique in that it spreads intraperitoneally, meaning within the abdominal (peritoneal) cavity. Ovarian cancer spreads when the malignant cells detach from the ovary and travel through intraperitoneal fluid, which in ovarian cancer, can contain abnormally accumulated ascites. Ascites that lead to abdominal swelling create a favorable environment for tumor growth and contain malignant cells that are then able to metastasize onto other abdominal organs and structures (Lheureux et al., 2019). Advanced stage disease is more difficult to treat due to the larger tumor burden, and survival is worse for these patients. When detected at an early stage, ovarian cancer is curable (Lheureux et al., 2019). This emphasizes the importance of cancer research to develop screening methods, which at this time are unavailable, in order to identify ovarian cancer earlier.

Types of Ovarian Cancer

Scientists have organized ovarian cancer into three different groups: epithelial, sex cord-stromal, and germ cell neoplasms. The most common type is epithelial-derived ovarian cancer, which accounts for over 90% of all occurrences (N. Zhang et al., 2017). Even though most ovarian cancers are detected in the ovary, many of them do not actually originate in ovarian tissue.

This phenomenon is observed especially in epithelial-derived ovarian cancers, which is discussed below (Diep et al., 2015). Germ cell neoplasms originate from primordial germ cells and occur in 5-10% of all ovarian cancers (Kaur, 2020). Finally, sex cord-stromal ovarian cancer is the rarest and are derived from non-germ cell components, such as granulosa cells (N. Zhang et al., 2017).

Epithelial-Derived Ovarian Cancer

Epithelial ovarian cancer (EOC) is the largest group of ovarian cancers and is heterogeneous in nature because of possible differences observed in histology presentation, tumor grade, and molecular features (Lheureux et al., 2019). As mentioned earlier, many of these cancers originate from metastasized non-ovarian cells that migrate and settle in the ovary (Diep et al., 2015). Based on histological presentation, EOCs are divided into five different subtypes. High-grade serous ovarian cancer (HGSOC) accounts for approximately 70% of EOCs, making it the most common subtype of EOC (Lheureux et al., 2019). Usually, this EOC originates in the fallopian tubes before migrating to form cancer in ovarian tissue (Diep et al., 2015). HGSOCs can occur sporadically, but there are genetic associations in cancer development as well. Two of the most noted genes associated with this subtype are *BRCA* genes and *TP53*. *BRCA1* and *BRCA2* genes are known tumor suppressors involved in genome stability and DNA repair processes. *BRCA* gene mutations are also linked with breast cancer, and patients with a familial history of this disease may be more predisposed to the development of ovarian cancer. *TP53* regulates expression of genes that affect processes such as DNA repair and the cell cycle. Along with genetic characteristics, the histological presentation of HGSOCs features solid, papillary tumor growth and large cells containing pleiomorphic nuclei. Low-grade serous ovarian cancer (LGSOC) represents another subtype of EOC. Histologically, this subtype features smaller papillae with nuclei of a similar size and shape. The most commonly mutated genes associated with LGSOCs are oncogenes *BRAF* and *KRAS.* Also, researchers have noticed activation of a signaling pathway known as the mitogen-activated protein kinase (MAPK) pathway involved in causing many LGSOCs. Tied with LGSOC at 10% of cases, endometrioid ovarian cancer is another member of the EOC family. Imaging portrays this form of EOC as cystic and solid. Mutations in genes such as *PIK3CA*, *ARID1A*, *KRAS*, *PTEN*, and *PPP2R1A* affect a series of signal transduction pathways, possibly leading to increased chance of endometrioid cancer formation. Making up around 5% of EOCs, ovarian clear cell carcinoma (OCCC) represents another subtype. This group features histology with complex papillae formation and cell

cytoplasm uniquely filled with glycogen. Currently, researchers have found three distinct signaling pathways that, when the respective genes regulating them are mutated, can potentially lead to OCCC development. Similar to endometrioid cancer, a potential gene mutation implicated in OCCC is *PIK-3CA*. This gene works to regulate the PI3k/Akt signaling pathway, and when mutations of the gene occur, this could cause overactivation of the pathway (Lheureux et al., 2019). The PI3K/Akt signaling mechanism is utilized by many cancer types because the pathway helps to increase processes, such as proliferation and survival for the cells (Gocher et al., 2017). The *ARID1A* gene is a member of a complex of genes affecting chromatin remodeling, and mutations of it can lead to OCCC cancer formation. Finally, receptor tyrosine kinase (RTK)/Ras signaling pathways can also be implicated in causing OCCC if gene mutation occurs (Lheureux et al., 2019). Research has found OCCC and endometrioid cancers are similar in their origin, typically the cervix or endometrium. In fact, both of these cancer subtypes have higher prevalence in those with a history of endometriosis (Diep et al., 2015). The final subtype is mucinous ovarian cancer, which is the rarest form of EOC. Gene mutations found implicated in causing mucinous ovarian cancer are those in *KRAS* and *HER2*. These mutations can lead to abnormal cell growth, contributing to cancer formation. This form of cancer is unique microscopically: It exhibits multiple-compartmented cysts filled with a mucous fluid. It is also heterogenous in nature—possessing both invasive and noninvasive features (Lheureux et al., 2019). Most of the invasive species originate from organs in the lower intestinal tract such as the colon (Diep et al., 2015). As discussed, there are many presentations of EOCs and even more potential molecular features that when dysregulated could lead to abnormal cell growth or cancer. This variety reinforces why there has been difficulty in identifying and regulating cancer formation.

Germ Cell Neoplasms

Rarer than EOCs, germ cell neoplasms come from primitive germ cells and can be divided into two large groups: mature cystic teratomas, which are usually benign and most common, and malignant ovarian germ cell tumors (MOGCT) (Kaur, 2020). The mature cystic teratomas contain tissues derived from the embryonic layers of ectoderm, mesoderm, and endoderm. Although normally benign, this form of neoplasm can transform into malignant tissue, but this usually does not occur until older adulthood. MOGCTs most often affect younger females, who will start to experience severe abdominal pain with a large mass in the abdominal region. This differs from epithelial-derived ovarian tumors that normally affect older females, especially those over

the age of 65 years old. Based on imaging, MOGCTs usually contain solid, cystic elements and can feature teratoma components. With proper diagnosis, patients with this form of tumor can have better outcomes than with other ovarian cancers, especially EOCs, but as with most other cancers, a later diagnosis can mean a poorer prognosis (Shaaban et al., 2014).

Sex Cord-Stromal Ovarian Cancer

Sex cord-stromal ovarian cancers attain their derivation from components that are non-germ cells. This can include granulosa cells that have supportive and regulatory functions for germ cells (N. Zhang et al., 2017). This form of ovarian cancer usually presents itself in early adulthood and displays abnormal hormone production. The tumors can vary in firmness and texture, and the cells are usually pleiomorphic in shape. Molecular abnormalities can be found, typically caused by mutations in the genes *DICER1*, *STK11*, and *FOXL2*. DICER1 expression regulates pathways involved in microRNA processing. *STK11* mutations might be related to abnormal progesterone secretion contributing to cancer formation. Finally, the *FOXL2* gene, when mutated, is known to decrease the rate of apoptosis in cells and limit function of pathways that prevent proliferation of cancerous cells (Schultz et al., 2016). Also, mutations in this gene can lead to increases in estrogen production, which can be associated with promoting cancer formation (Langdon et al., 2020). A better understanding of the potential genes, signaling pathways, and hormones involved in ovarian cancer formation could help researchers and clinicians understand the cause of ovarian cancer and develop more effective, targeted therapies.

Hormones Associated with Healthy Ovaries

Ovarian function has a strong association with endocrine signaling, and there are several hormones that play a large role in regulating physiological mechanisms within the body. Two of the most well-known are steroid hormones derived from cholesterol: estrogen and progesterone. These endocrine molecules help control cellular processes such as apoptosis, proliferation, and differentiation (Lima et al., 2016). Each hormone influences unique signaling pathways through ligand-receptor interaction that promote a cascade of downstream effects.

Progesterone

Structure and Function

Progesterone, a member of the progestogen family of steroid hormones,

is active during the second half of the menstrual cycle. It is produced and secreted by the corpus luteum to provide an environment for an egg to be fertilized. After ovulation, if the egg is not fertilized, the corpus luteum will shed and progesterone levels will fall triggering a period (Lima et al., 2016; Richards & Pangas, 2010). During normal ovulation cycles, progesterone release regulates reproductive tissue differentiation, and during pregnancy, progesterone is present at high levels to ensure proper development of fetal tissues and breast tissues and to prepare the endometrium for implantation (Diep et al., 2015). Some research also shows that progesterone might play a role in preventing the formation of tumors, and progesterone therapies are used to treat certain types of cancer (Lima et al., 2016). In fact, decreases in progesterone levels, which can be caused by factors such as aging, can be associated with an increased risk for developing ovarian cancer (Diep et al., 2015). Ovarian function is dependent on the interaction between progesterone and its progesterone receptor (PR) to carry out roles such as ovulation control and luteinization (Lima et al., 2016). PRs exist primarily in three different isoforms: PR-A, PR-B and PR-C. PR-A and PR-B work as transcription factors to regulate gene expression when activated by their ligand, progesterone. PR isoform PR-C does not have a DNA binding domain, so it is unable to enact any function by binding to DNA, and its expression is limited in general. Because progesterone functions primarily in reproductive organs, most of the PRs can be found expressed in breast, uterine, and ovarian tissue in order to promote hormone signaling. It is important to note that the protective tendencies of progesterone seen in ovaries seems to be absent in breast cancers. In fact, progesterone plays a role in stem cell expansion in breast tissue. Estrogen presence induces expression of PR, which promotes signaling processes to increase cell proliferation, which can lead to tumorigenic effects in the breast. However, in ovarian tissue, progesterone function opposes that of estrogen, reducing proliferation of tissue and therefore protecting against ovarian cancer (Diep et al., 2015).

Signal Transduction Pathways

Progesterone signaling can enact its effects in two different ways: inside the nucleus or outside the nucleus. Intranuclear signaling might include progesterone action on cis elements of the DNA to induce transcription and increase gene expression. In order to reach the nucleus to exert its effects, progesterone is able to diffuse through the membrane of a cell, because of its nonpolar characteristics, to eventually bind to a PR. If functioning in an extracellular manner, progesterone can act as either an autocrine or paracrine signaling molecule and directly bind to enzymes to affect other signal trans-

duction pathways. One example would be ERK1/2 MAP kinase pathways. When progesterone binds, it can activate this pathway to begin phosphorylation of several transcription factors. Researchers have also found that progesterone signaling can be regulated itself through activation of this pathway because it can become phosphorylated by the ERK1/2 MAP kinase (Diep et al., 2015).

Estrogen

Structure and Function

Estrogen makes up another class of steroid hormones and is well-known for its role in cell proliferation and differentiation to promote female sexual characteristics (Hernández-Silva et al., 2020; Liu et al., 2017). There are three types of estrogen hormones: estrone, 17β-estradiol, and estradiol, which is the form most commonly thought of during a woman's childbearing years. The ovaries and adrenal glands both work to synthesize estrogen hormones that will act on two types of receptors known as estrogen receptor alpha (ERα) or estrogen receptor (ERβ), which are found in the nucleus and the cytoplasm (Hernández-Silva et al., 2020). Another estrogen receptor that has gained some interest recently in the scientific community is a G protein-coupled receptor known as G protein-coupled estrogen receptor 1 (GPER1). This is a membrane bound receptor that promotes the activity of several signal transduction pathways mentioned in the next section (Ribeiro & Freiman, 2014). Each of the three types of estrogen hormones act differently when bound to GPER. Research has shown that 17β-estradiol and estrone act as agonists for GPER to promote its effects, whereas estriol seems to work against GPER, displaying antagonistic behaviors (Hernández-Silva et al., 2020).

Signal Transduction Pathways

When estrogen interacts with ERα and ERβ, the signaling pathways that ensue are known as genomic signaling. Once estrogen has bound to one of these receptors, the complex can be translocated to the nucleus, where it can bind to estrogen response elements (EREs). This binding can either regulate transcriptional events or work to recruit other transcription factors to regulate transcription indirectly. Another form of signaling known as non-genomic estrogen signaling employs GPER to activate different signal transduction pathways involving kinase activity to carry out several biological mechanisms (Ribeiro & Freiman, 2014). One of the classic signal cascades that occurs as a result of GPER activation is an increase in cyclic AMP (cAMP) production, which activates protein kinase A (PKA). PKA activation assists with the transactivation of epidermal growth factor receptor (EGFR) as well as MAPK and

PI3K/Akt pathways. These pathways have kinase activity and work to regulate genes that are involved with processes such as cell survival, proliferation, and differentiation (Hernández-Silva et al., 2020). Understanding the downstream cascades estrogen signaling might induce can help expose systems that might be affected if dysfunctional signaling were to occur.

Hormone Signaling Linked to Ovarian Cancer

Many endocrine signaling pathways are involved in regulating ovarian function and promoting overall female reproductive health. However, because there are so many pathways involved, there could be more places where malfunction could occur, which could lead to abnormal cellular processes that contribute to tumorigenicity and ovarian cancer. Although the main focus will be on progesterone and estrogen's dysfunction to promote ovarian cancer, there are some additional female hormones that are associated that can be observed.

Progesterone

As mentioned before, researchers have found progesterone seems to help prevent ovarian cancer formation. This can be illustrated by observing the effects oral contraceptives have on cancer risk. Because oral contraceptives increase progesterone levels to decrease ovulation, studies show that use of these medications can decrease risk of cancer by 50% after five years of use. Progesterone's protective effects can also be observed during pregnancy when hormone levels are similarly significantly increased. Additionally, clinicians have been able to identify PR expression in cancerous ovarian tissue, and they find this to improve prognostic outcomes. Those with increased expression of PRs saw an improvement in survival (Diep et al., 2015).

Studies have observed the PR distribution within ovarian cancers, specifically EOCs, to see its effect on tumor progression and prognosis. One study found that in 35% of ovarian tumors, expression of PR was increased. In fact, EOCs with the highest PR expression were endometrioid followed by LGSOC and HGSOC. This positive PR expression was shown to improve patient survival as progesterone seemed to downregulate cancer cell processes such as proliferation, DNA synthesis, and cell division (Diep et al., 2015; Luo et al., 2017). For example, when at least 50% of tumor nuclei-stained cells were expressing PR, this correlated with an increase in survival outlook. Most of the PRs that were expressed in these cancers were the PR-B isoform. However, there were relatively small expression levels of PR-A expression within mucinous ovarian cancer, LGSOCs, and HGSOCs (Diep et al.,

2015). While observing some of these studies, it should be noted that much of the positive PR-expression is generally found in borderline tumors, which are considered atypical and proliferative but non-cancerous as malignant tumors (93%) have been shown to have little to no PR expression, which gives further evidence to the idea that progesterone signaling has a tumor suppressive action (Luo et al., 2017).

Although the mechanism of progesterone and PR action in slowing cancer progression is relatively unknown, one study from the University of Minnesota detailed a potential pathway involving a transcription factor called FOXO1. This molecule interacts with PRs bound by progesterone to induce cellular senescence, a process in which the cells are growth-arrested. When treated with progesterone, FOXO1 and progesterone-PR form a complex at a cis-acting sequence of DNA to increase expression of a gene called cyclin-dependent kinase inhibitor p21, which is involved in arresting the cell cycle to suppress tumor progression. However, the study also showed FOXO1-PR-progesterone action is sensitive to kinase activity. As mentioned previously, one of the pathways that regulates progesterone signaling is the MAPK signaling pathway. This study observed that both FOXO1 and PR phosphorylation, due to increased kinase activity in ovarian cancers, prevented them from entering the nucleus and targeting tumor suppressive genes, respectively. The decreased activity of transcription factor FOXO1 seemed to be linked to tumor generation and cancer progression (Diep et al., 2015). Although there have been recent discoveries regarding progesterone's role in tumor progression, researchers have much more to uncover in order to find more potential treatment targets.

Estrogen

While progesterone has been shown to be tumor suppressive, estrogen has been shown to promote tumorigenesis. In one study, researchers found that aggressive, malignant ovarian cancers showed positive expression levels of ER (estrogen receptor). Interestingly, of the three types of ERs discussed previously, ERα and GPER expression seems to promote tumor growth, whereas ERβ might decrease tumor growth. Usually when the ratio of ERα to ERβ was higher, there was a greater occurrence of malignant ovarian cancer (Ribeiro & Freiman, 2014). Some studies show GPER promotes oncogene activity. Higher levels of GPER expression were observed, especially in malignant ovarian tumors. This increased expression was found to be linked to poorer patient outcomes. When expression of GPER was decreased in cancer cells, this correlated with increased survival rates (Hernández-Silva et al., 2020). It is important to highlight, however, that GPER's role in regulating

ovarian tumorigenesis is controversial. GPER tumor activating properties could be tissue dependent, which is detailed by one study that observed decreased expression of GPER in breast cancer cell lines compared to healthy cells. This study also found that when GPER had an agonist bound ovarian cancer cell, proliferation was decreased due to apoptotic activity and arrested cell cycles (Ignatov et al., 2013). There could be several factors that influence whether GPER acts in a suppressive or oncogenic manner. It could be based on activation of separate pathways, a difference in signaling molecule interaction, or exposure to different exogenous compounds. These contradicting results among estrogen signaling with different ERs further the notion that more investigation must be pursued to understand the underlying causes and predictive markers associated with ovarian cancer.

Several estrogen signaling pathways have been implicated in promoting ovarian cancer. The MAPK signaling pathway is one example. It functions closely with an isoform of ERα that is ligand-independent and activates signal transduction pathways other than those of genomic signaling mentioned before. MAPK uses kinase activity to phosphorylate a residue on ERα, causing it to activate. In some ovarian cancers, this signaling pathway becomes overactive, which can lead to an ERα resistance to anti-estrogen therapies, which are a treatment for ovarian cancers. This resistance occurs because overactivation of ERα causes the receptor to have a higher affinity for estrogen and lesser affinity for the anti-estrogen compounds. However, when MAPK activity is inhibited, decreased responses to anti-estrogen can be reversed, and normal estrogen signaling can be restored (Ribeiro & Freiman, 2014). The PI3K/Akt pathway is another mechanism possibly implicated in ovarian tumorigenesis. As mentioned before, GPER can activate PI3K/Akt signaling to lead to cellular events, such a proliferation, which can be devastating when applied to cancer cells. As with much of ovarian cancer research, information on the effect of PI3K/Akt signaling on ovarian cancer cells is limited. However, in breast cancer cells, PI3K activation led to cancer cell proliferation and resistance to hormone therapies. Whenever the researchers were able to inhibit the pathways, endocrine resistance was restored and much of the abnormal signaling that promoted proliferative properties were reversed. Although we know PI3K/Akt activation is linked to estrogen signaling in ovarian cancer cells, more research needs to be conducted to conclude whether the pathway has the same effects of building endocrine resistance in these cells versus breast cancer cells. Additionally, the effects of inhibiting the pathway in ovarian cancer cells is relatively unknown (Ribeiro & Freiman, 2014). While ERα and GPER seem to influence oncogenic capabilities, research shows ERβ works in the opposite way, promoting tumor suppression. Patients who

express higher levels of the receptor have a better prognosis and a reduced chance of cancer recurrence after treatment. Additionally, these patients tend to respond better to platinum-based chemotherapy treatments and have decreased chance of drug resistance. ERβ promotes its anti-tumorigenic effects by reducing activity of pathways, such as PI3K/Akt, and increasing expression of the tumor suppressive gene, p21 (Pinton et al., 2018).

GnRH, FSH, and LH

While estrogen and progesterone are two of the most well-known hormones with significant roles in female reproductive health, there are other hormones that also have endocrine function that when manipulated, can potentially cause ovarian cancer. One of these hormones, which was mentioned previously, is GnRH. It acts as a tropic hormone to stimulate the release of hormones called FSH and LH. Observations during research have led scientists to believe GnRH's receptor (GnRHR) might act as a tumor suppressor. They saw that most patients experienced better survival rates if their ovaries had increased GnRHR expression. GnRHR also mediates antiproliferative effects through activation of protein kinase C (PKC), which activates the ERK1/2 pathway through phosphorylation. When GnRHR was blocked, this pathway was downregulated, and proliferation of cancer cells increased. Interestingly, in relation to estrogen and its potential tumorigenic effects, estrogen signaling increases caused a decrease in GnRHR expression, which resulted in increased proliferation from ovarian cancer cells (Q. Zhang et al., 2017).

One of the hormones released as a result of GnRH signaling is FSH. Abnormal FSH signaling can lead to a host of malignant tumor characteristics such as invasiveness, cell migration, and growth. Increases in FSH expression levels were associated with reduced expression in tumor suppression genes, such as BRCA1, and higher tumor grades. Generally, FSH induces these mechanisms through ligand interaction with FSH receptor (FSHR), which leads to activation of PI3K/Akt and ERK1/2 pathways to increase proliferative and invasive effects of cancer cells. However, the opposite effect occurred when FSH signaling was downregulated by FSH inhibitors (Q. Zhang et al., 2017).

Closely related to FSH is LH, which is the other gonadotropic hormone released as a result of GnRH stimulation. Some studies have shown LH can reverse the tumor causing effects induced by FSH. One unique aspect of LH expression is that when upregulated, it can cause an increase in expression among miRNAs that help promote antiproliferative processes. LH can also curb invasive and proliferative aspects of ovarian cancer cells by upregulating

proteins that increase cell adhesion, a characteristic that is lost with cancerous cells and tissue (Q. Zhang et al., 2017). By understanding the signaling patterns of these reproductive hormones, researchers can gain important insight on potential targets for ovarian cancer treatments.

Hormone-Related Cancer Treatment Targets

Developing a cure for cancer has been on the forefront of research in the scientific community for many years. While cancer treatments and rates of survival have increased significantly over the past several years, there are still many unknown variables and barriers researchers have yet to pass. As mentioned before, research related to hormone involvement in ovarian cancer is still somewhat obscure. However, studies have been conducted in recent years that have observed female reproductive hormones and some of the signaling pathways they influence as potential targets for ovarian cancer treatment.

One potential target for ovarian cancer treatment is ERβ. Unlike GPER and ERα, research has shown that ERβ expression can be associated with tumor suppressive effects by activating pathways associated with tumor downregulation and decreasing contents of molecules in the cells that increase tumor cell signaling (Pinton et al., 2018). For this reason, scientists have performed experiments to capitalize on ERβ's protective effects by developing agonists to increase activity of the receptor. One particular study shows natural ERβ agonists can mitigate some platinum-based therapeutic resistance of cancer cells by bringing back sensitivity of the cells to chemotherapy drugs when the agonist is used in combination with them. Also, they found that the ERβ agonists are able to enhance the effects of ERβ, which led to decreases in tumor properties, such as cancer cell migration and invasion. Furthermore, staining and imaging results of these experiments also showed cancer cells treated with ERβ agonist result in increases in cancer cells that are apoptotic and decreases in expression of cellular markers for proliferation. Finally, when analyzing and comparing RNA sequencing data of cancer cells without ERβ agonist treatment with data of cancer cells with treatment, there were decreases in expression of genes involved in functions, such as cellular growth and proliferation when treated with the natural agonist (Liu et al., 2017).

The MEK signal transduction pathway is a potential target for treatment as well. This mechanism can increase expression of genes that regulate ERα signaling. One of these regulatory genes is TFF1, and research shows increased expression of this gene helps improve response to anti-estrogen therapies. Aside from decreasing ERα expression, when the MEK inhibitor is combined with other chemotherapy drugs, the inhibitor has been shown to

also reduce expression of genes, such as EGFR and HER2 that produce cell proliferation and growth effects (Ribeiro & Freiman, 2014). There are many signaling pathways involved in controlling ovarian cell processes, which were mentioned earlier. If these mechanisms malfunction, this can lead to cancer formation and resistance to some therapies. Despite this, researchers have recently been using their knowledge of these pathways to develop treatments to inhibit or target them in some way to help treat ovarian cancer, as we see with the MEK inhibitor.

Genetics can be a significant indicator of ovarian cancer susceptibility. In fact, about 20% of ovarian cancer incidence is found to be caused by inherited genetic abnormalities. The BRCA genes are especially significant indicators in patient outcome. These genes are tumor suppressors involved in DNA repair pathways, so when damaged either by abnormal signaling, pathway dysfunction, or environmental factors, cells can have a higher mutation rate and lead to an increased risk for cancer. One drug therapy shown to help treat ovarian cancer in patients with these genetic mutations are poly (ADP-ribose) polymerase (PARP) inhibitors. PARP enzymes help repair DNA, specifically single-strand breaks. When the enzymes are targeted with PARP inhibitors, single strand breaks in cellular DNA are not repaired, and this can lead to double-stranded break accumulation. Patients with BRCA gene mutations are sensitive to PARP inhibitor action because with treatment, there are more DNA repair pathways interfered with, causing increased accumulation of DNA damage and cell death in cancerous cells. One PARP inhibitor that has shown success in treating ovarian cancer is Olaparib. This drug increases chances of progression-free survival, decreased platinum-based chemotherapy resistance, and an overall better prognosis among those with ovarian cancer (Lheureux et al., 2019).

Another potential target for hormone regulation is by using aromatase inhibitors. These compounds work to prevent the transition of estrogen from androgens. By doing this, the amount of estrogen in the body is decreased. This means there is less ligand-receptor interaction between ERα, or other estrogen receptors, and estrogen, which limits potential tumorigenic activity that can be associated with this receptor. Another drug that prevents estrogen activity is tamoxifen, which is well-known in breast cancer treatment. This treatment shows comparable ability to the aromatase inhibitors in stabilizing disease with only a 4% difference between the two. One of the largest barriers to effectiveness of anti-estrogen treatments for ovarian cancers is the drug resistance that cancer cells can develop. Although these treatments provide viable options for cancer patients, drug effectiveness might fluctuate throughout treatment of the cancer, and responses may vary case by case

(Langdon et al., 2020). This furthers the point that more research needs to be performed to understand these cellular processes involved with cancer's development and progression and how ovarian cancer cells are able to adapt to their environment to build chemotherapy resistance. Understanding more about hormones, their signaling pathways, and how malfunction can lead to ovarian cancer will help scientists develop more treatment targets to hopefully improve disease outlook for ovarian cancers.

Conclusion

Although cancer research has made tremendous strides to develop useful screening tests in other fields, unfortunately ovarian cancer lags behind. Ovarian cancer is diagnosed at an advanced stage, and treatment involves removing as much of the cancer as possible with surgery followed by platinum-based chemotherapy. Despite intense treatments, most patients will experience recurrence and succumb to the disease. Therefore, understanding the cause of ovarian cancer and developing more effective screening tools and therapies is imperative to improve the survival for such patients. Professionals have an in-depth knowledge about the overall structure and function of the ovaries to produce female germ cells for fertilization and secrete important reproductive hormones such as estrogen and progesterone. These hormones function to regulate processes such as ovulation, pregnancy, cell differentiation, and the development of sex characteristics. In order to regulate these processes, progesterone and estrogen interact with their respective receptors to allow activation of several different signal transductions mechanisms including MAPK and PI3K pathways. If there is malfunction of these signaling mechanisms, it can lead to development of a series of ovarian cancers that fall into three categories: EOC, germ cell neoplasms, and sex cord-stromal ovarian cancers. Researchers have linked abnormal hormone regulation of estrogen, progesterone, GnRH, LH, and FSH with tumorigenic effects such as proliferation, cell invasion, and anti-apoptosis. However, there is still much uncertainty on some of the pathways these hormones act upon and how they are implicated in promoting ovarian cancer. As researchers look into these mechanisms and begin to understand more about the downstream effects of these signaling pathways, they are also learning about ways they can target these hormones and signaling pathways. Hopefully, investigators can use these discoveries to identify novel therapeutic targets and develop new treatments that may help slow the progression of ovarian cancer, increase effectiveness of chemotherapeutic agents, and ultimately improve survival in this patient population.

References

Diep, C. H., Daniel, A. R., Mauro, L. J., Knutson, T. P., & Lange, C. A. (2015). Progesterone action in breast, uterine, and ovarian cancers. *Journal of Molecular Endocrinology, 54*(2), R31–R53. https://doi.org/10.1530/JME-14-0252

Gocher, A. M., Azabdaftari, G., Euscher, L. M., Dai, S., Karacosta, L. G., Franke, T. F., & Edelman, A. M. (2017). Akt activation by Ca2+/calmodulin-dependent protein kinase kinase 2 (CaMKK2) in ovarian cancer cells. *The Journal of Biological Chemistry, 292*(34), 14188–14204. https://doi.org/10.1074/jbc.M117.778464

Hernández-Silva, C. D., Villegas-Pineda, J. C., & Pereira-Suárez, A. L. (2020). Expression and role of the G Protein-Coupled Estrogen Receptor (GPR30/GPER) in the development and immune response in female reproductive cancers. *Frontiers in Endocrinology, 11,* 544. https://doi.org/10.3389/fendo.2020.00544

Ignatov, T., Modl, S., Thulig, M., Weißenborn, C., Treeck, O., Ortmann, O., ... Ignatov, A. (2013). GPER-1 acts as a tumor suppressor in ovarian cancer. *Journal of Ovarian Research, 6*(1), 51. https://doi.org/10.1186/1757-2215-6-51

Kaur, Baljeet (2020). Pathology of malignant ovarian germ cell tumours. Diagnostic *Histopathology, 26*(6), 289–297. https://doi.org/10.1016/j.mpdhp.2020.03.006

"Key Statistics for Ovarian Cancer" (2021). *American Cancer Society*. https://www.cancer.org/cancer/ovarian-cancer/about/key-statistics.html

Langdon, S. P., Herrington, C. S., Hollis, R. L., & Gourley, C. (2020). Estrogen signaling and its potential as a target for therapy in ovarian cancer. *Cancers, 12*(6), 1647. https://doi.org/10.3390/cancers12061647

Lheureux, S., Braunstein, M., & Oza, A. (2019). Epithelial ovarian cancer: Evolution of management in the era of precision medicine. *CA: A Cancer Journal for clinicians, 69*(4), 280–304. https://doi.org/10.3322/caac.21559

Lima, M.A., da Silva, S.V. & Freitas, V.M. (2016). Progesterone acts via the progesterone receptor to induce adamts proteases in ovarian cancer cells. *Journal of Ovarian Research, 9*(9). https://doi.org/10.1186/s13048-016-0219-x

Liu, J., Viswanadhapalli, S., Garcia, L., Zhou, M., Nair, B. C., Kost, E., ... Sareddy, G. R. (2017). Therapeutic utility of natural estrogen receptor beta agonists on ovarian cancer. *Oncotarget, 8*(30), 50002–50014. https://doi.org/10.18632/oncotarget.18442

Luo, H., Li, S., Zhao, M., Sheng, B., Zhu, H., & Zhu, X. (2017). Prognostic value of progesterone receptor expression in ovarian cancer: a meta-analysis. *Oncotarget, 8*(22), 36845–36856. https://doi.org/10.18632/oncotarget.15982

Pinton, G., Nilsson, S., & Moro, L. (2018). Targeting estrogen receptor beta (ERβ) for treatment of ovarian cancer: importance of KDM6B and SIRT1 for ERβ expression and functionality. *Oncogenesis, 7*(2), 15. https://doi.org/10.1038/s41389-018-0027-9

Ribeiro, J. R., & Freiman, R. N. (2014). Estrogen signaling crosstalk: Implications for endocrine resistance in ovarian cancer. *The Journal of Steroid Biochemistry and Molecular Biology, 143,* 160–173. PMCID: PMC4127339

Richards, J. S., & Pangas, S. A. (2010). The ovary: basic biology and clinical implications. *The Journal of Clinical Investigation, 120*(4), 963–972. https://doi.org/10.1172/JCI41350

Schultz, K. A. P., Harris, A. K., Schneider, D. T., Young, R. H., Brown, J., Gershenson, D. M., ... Frazier, A. L. (2016). Ovarian sex cord-stromal tumors. *Journal of Oncology Practice, 12*(10), 940–946. https://doi.org/10.1200/JOP.2016.016261

Shaaban, A.M., Rezvani, M., Elsayes, K.M., Baskin H. Jr., Mourad, A., Foster, B.R., ... Menias, C.O. (2014). Ovarian malignant germ cell tumors: cellular classification and clinical and imaging features. *Radiographics, 34*(3), 777–801. https://doi.org/10.1148/rg.343130067

Zhang, N., Chen, R., Hua, K., & Zhang, Y. (2017). A retrospective study of

reproductive outcomes after fertility-sparing surgery and postoperative adjuvant chemotherapy in malignant ovarian germ cell tumors and sex cord-stromal tumors. *Journal of Ovarian Research, 10*(1), 52. https://doi.org/10.1186/s13048-017-0348-x

Zhang, Q., Madden, N. E., Wong, A.T. S., Chow, B. K. C., & Lee, L. T. O. (2017). The role of Endocrine G Protein-Coupled Receptors in ovarian cancer progression. *Frontiers in Endocrinology, 8,* 66. https://doi.org/10.3389/fendo.2017.00066

Effects of Autogenic Training on Stress Reduction and Glycemic Control on a College Student with Type 1 Diabetes Mellitus

Tyler Swearingin and Rachel Ingram

Abstract

Stress is an indicator for poor glycemic control in the college-aged type 1 diabetic population. It was hypothesized by the researchers in this study that by introducing an autogenic training program, a form of relaxation training, into a sample of college students with type 1 diabetes mellitus, a reduction in stress and an improvement in glycemic control would result. The present case study consisted of one 18-year-old female participant. Measures of stress include the Diabetes Distress Scale, the Depression Anxiety Stress Scale, and the Problem Areas In Diabetes scale. Glycemic control will be measured using blood glucose levels. A single-subject design case study implemented the daily use of autogenic training after a baseline measurement. The primary dependent measure is the mean recordings of the participant's daily blood glucose levels, and scores from the psychological tests serve as a supplemental measure to indicate changes in stress and well-being. Researchers used statistical and visual analysis to interpret the results of the study, and improvement of glycemic control was found, although no psychological measures showed a positive improvement except the Diabetes Distress Scale, which showed improvement in all subscales. Implications include the future use of autogenic training as a promising method to reduce diabetes-related distress and improve glycemic control in college students with type 1 diabetes mellitus.

Introduction

Diabetes mellitus is a prevalent disease around the world, particularly in the United States. Diabetes mellitus is classified as a chronic metabolic disease,[1,2] and as of 2014, affected an estimated 422 million adults worldwide.[3] Type 1 diabetes mellitus (T1DM) is an autoimmune disorder that prevents an individual from producing insulin and is diagnosed at a much younger age than type 2 diabetes mellitus (T2DM). In contrast, T2DM is caused by either an insulin deficiency or a reduction in insulin sensitivity.[4] Those diagnosed with T1DM experience both microvascular complications such as neuropathy, nephropathy, and retinopathy, as well as macrovascular complications such as coronary artery disease, peripheral arterial disease, and stroke.[1,2]

Most of the complications that come with a T1DM diagnosis are due to chronic hyperglycemia, a symptom of poor glycemic control.[5] The prevalence of poor glycemic control is around 25% for adult patients with T1DM,[6] and Geddes, McGeough, and Frier detail glycemic control is even more difficult for young adults going through college,[7] possibly due to schedules, increased work requirements, social factors, etc. With a high incidence for hyperglycemia and poor glycemic control, it is necessary for all patients with T1DM to implement an intensive insulin therapy program into their lives. Therapy aims to achieve "better" glycemic control, but in many patients, it has instead turned the reduced risk of hyperglycemia into an increased risk of hypoglycemia.[8]

Management approaches such as physical activity, diet, and weight loss have all been used as methods to improve glycemic control, but these factors, along with the medical requirements of the disease, contribute to the overall stress of those with T1DM.[9] In fact, Li and colleagues[10] found the prevalence of severe psychological distress in adults with diabetes to be 7.6%. Another

1 Fowler, M. J. 2008. Microvascular and macrovascular complications of diabetes. *Clinical Diabetes, 26*(2): 77–82.
2 Porte, D., & Schwartz, M. W. 1996. Diabetes complications—Why is glucose potentially toxic? *Science, 272* (5262): 699.
3 World Health Organization. 2021. Fact sheets: diabetes.
4 McCrimmon, R. J., Ryan, C. M., & Frier, B. M. 2012. Diabetes and cognitive dysfunction. *Lancet, 379:* 2291–2299.
5 Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England Journal of Medicine, 329:* 977–986.
6 Devries, J. H., Snoek, F. J., & Heine, R. J. 2004. Persistent poor glycaemic control in adult type 1 diabetes. A closer look at the problem. *Diabetic Medicine, 21*(12): 1263–1268.
7 Geddes, J., McGeough, E., & Frier, B. M. 2006. Young adults with type 1 diabetes in tertiary education: do students receive adequate specialist care? *Diabetic Medicine, 23*(10): 1155–1157.
8 Diabetes Control and Complications Trial Research Group.1997. Hypoglycemia in the diabetes control and complications trial. *Diabetes, 46:* 271–286.
9 Noordali, F., Cumming, J., & Thompson, J. L. 2017. Effectiveness of mindfulness-based interventions on physiological and psychological complications in adults with diabetes: a systematic review. *Journal of Health Psychology, 22*(8): 965–983.
10 Li, C., Ford, E. S., Zhao, G., Strine, T. W., Dhingra, S., Barker, L., ... Mokdad, A. H. 2009. Association between diagnosed diabetes and serious psychological distress among U.S. adults: the Behavioral Risk Factor Surveillance System, 2007. *International Journal of Public Health, 54* (Suppl 1): 43–51.

study found 30% of patients with T1DM identified factors associated with correcting poor glycemic control as a cause of distress.[11] Other studies have found diabetes to be related to two times the risk of developing depression[12] and a 40% probability of developing increased anxiety symptoms.[13]

Another cause of increased distress may be the stigma associated with the disease. Schabert, Brown, Mosely, and Speight (2013) found in studies quoted in Shiu and others and in Broom and Whittaker that many people diagnosed with diabetes believe it brings about unwanted attention and the perception that others see them as a drug user. They note this stigma causes individuals with T1DM to develop distress, which deters them from providing adequate self-management of their diabetes. The effect of stress on diabetes is also physiological. Hormones such as cortisol, epinephrine, norepinephrine, glucagon, and insulin have all been found to be significantly affected by stress; each of these hormones is also directly related to carbohydrate metabolism and blood glucose levels.[14] Two different meta-analyses support this implication: One concluded out of 72 cases of diabetic ketoacidosis, 10 were attributed to psychological factors such as stress.[15] Another concluded out of 73 cases of diabetic ketoacidosis, 27% were due to the omission of insulin, half of which were caused by emotional factors such as stress and anxiety.[16]

The relationship between psychological stress and glycemic control, defined in this study as the proximity of the average of a patient's blood glucose readings to the nondiabetic range of 68–126 mg/dL, for individuals diagnosed with diabetes is evident because stress can cause higher HbA1c levels[17] and the indirect effect of disruption in dietary, activity, and insulin therapy regimen compliance.[18] For college students, this stress, if intensified or prolonged, may cause the other psychological symptoms of anxiety and depression, which were previously discussed regarding diabetics.[19]

Consequently, it is evident college students diagnosed with T1DM would benefit from stress reduction techniques in order to help relieve the effects of

11 Hislop, A. L., Fegan, P. G., Schlaeppi, M. J., Duck, M., & Yeap, B. B. 2008. Prevalence and associations of psychological distress in young adults with type 1 diabetes. *Diabetic Medicine, 25:* 91–96.
12 Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. 2001. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care, 24*(6): 1069–1078.
13 Grigsby, A. B., Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. 2002. Prevalence of anxiety in adults with diabetes: a systematic review. J*ournal of Psychosomatic Research, 53*(6): 1053–1060.
14 Surwit, R. S., Scovern, A. W., & Feinglos, M. N. 1982. The role of behavior in diabetes care. *Diabetes Care, 5*: 337–342.
15 Nabarro, J. D. N. 1965. Diabetic acidosis: clinical aspects. *On the nature and treatment of diabetes*: 545–557.
16 Cohen, A. S., Vance, V. K., Runyan, J. W. Jr, & Hurwitz, D. 1960. Diabetic acidosis: an evaluation of the cause, course, and therapy of 73 cases. *Annals of Internal Medicine,52*(1): 55–86.
17 Lloyd, C. E., Dyer, P. H., Lancashire, R. J., Harris, T., Daniels, J. E., & Barnett, A. H. 1999. Association between stress and glycemic control in adults with type 1 (insulin-dependent) diabetes. *Diabetes Care, 22*(8): 1278–1283.
18 Cox, D. J., Taylor, A. G., Nowacek, G., Holley-Wilcox, P., Pohl, S. L., & Guthrow, E. 1984. The relationship between psychological stress and insulin-dependent diabetic blood glucose control: preliminary investigations. *Journal of Health Psychology, 3*(1): 63–75.
19 Chang, S. B., Kim, S. Y., Oh, S. K., Yoo, I. Y., Kim, H. S., Kim, S., ... Choi, E. K., (2007). The effects of guided imagery program on high school male students' stress, stress coping, and health problems. J*ournal of Korean Academy of Psychiatric and Mental Health Nursing. 16*(1): 41–50.

stress and other psychological burdens associated with poor glycemic control. There are two primary methods of coping with stress: problem-focused and emotion focused. Emotion-focused stress reduction, which uses psychological and behavioral methods, has empirical support regarding stress reduction in college students.[20] Previous research has found stress-management interventions demonstrated effectiveness to reduce distress and improve glycemic control in individuals with diabetes.[21] One study showed motivational enhancement therapy, a systematic intervention approach based on principles of motivational psychology, is designed to produce internally motivated change rapidly.[22] Implemented over a 12-month time frame, it produced a limited 0.19% decrease in HbA1c,[23] while another study found cognitive behavioral therapy created a 0.94% decrease in HbA1c after only 24 weeks.[24] Behavioral medicine interventions have shown improvements in HbA1c, self-management, and both psychological and medical outcomes.[25]

However, one emotion-focused coping method, autogenic training, has less research on its effects on individuals with diabetes. Autogenic training falls within a specific branch of psychological therapy known as relaxation training. Relaxation training techniques have been efficacious in managing both stress and anxiety[26] and decreasing plasma levels of cortisol, the physiological indicator of increased stress.[27] These results suggest the use of autogenic training as a therapy for individuals with T1DM may be beneficial as it has been found to be the most comprehensive method of relaxation training, and it has been used as a clinical model for other relaxation training methods in the treatment of a variety of psychosomatic disorders.[28]

Autogenic training is a psychophysiological approach to psychotherapy designed primarily to enhance the individual's self-regulatory mechanism responsible for self-normalizing processes. In order to accomplish this,

20 Folkman, S., & Lazarus, R. S. 1980. An analysis of coping in a middle-aged community sample. *Journal of Health and Social Behavior, 21*(3): 219–239.
21 Surwit, R. S., van Tilburg, M. A., Zucker, N., McCaskill, C. C., Parekh, P., Feinglos, M. N., ... Lane, J. D. 2002. Stress management improves long- term glycemic control in type 2 diabetes. *Diabetes Care, 25*(1): 30–34.
22 Miller, W. R., Zweben, A., DiClemente, C. C., & Rychtarik, R. G. (1994). Motivational enhancement therapy manual: A clinical research guide for therapists treating individuals with alcohol abuse and dependence. Rockville, MD: NIAAA.
23 Ismail, K., Thomas, S. M., Maissi, E., Chalder, T., Schmidt, U., Bartlett, J., ... Treasure, J. 2008. Motivational enhancement therapy with and without cognitive behavioral therapy to treat type 1 diabetes patients: a randomized trial. *Annals of Internal Medicine, 149*(10): 708–719.
24 Amsberg, S., Anderbro, T., Wredling, R., Lisspers, J., Lins, P., Adamson, U., & Johansson, U. 2009. A cognitive behavior therapy- based intervention among poorly controlled adult type 1 diabetes patients—a randomized controlled trial. *Patient Education and Counseling, 77*(1): 72–80.
25 Plack, K., Herpertz, S., & Petrak, F. 2010. Behavioral medicine interventions in diabetes. *Current Opinion in Psychiatry, 23*: 131–138.
26 Kanji, N., White, A., & Ernst, E. 2006. Autogenic training to reduce anxiety in nursing students: randomized controlled trial. *Journal of Advanced Nursing, 53*(6): 729–735.
27 Jevning, R., Wilson, A. F., & Davidson, J. M. 1978. Adrenocortical activity during meditation. *Hormones and Behavior, 10*(1): 54–60.
28 Pelletier, K. R. 1979. "Holistic medicine: from pathology to prevention." *The Western Journal of Medicine, 131*(6): 481–483.

autogenic training methods are based on autosuggestion, a practice accomplished through six simple relaxation responses.[29] These responses start with the creation of passive concentration on heaviness in the extremities and then proceeds to the feeling of warmth in various parts of the body. Next, keeping the heartbeat calm and regular, breathing is slowed with deeper respirations. Lastly there is the feeling of warmth within the upper abdominal cavity and the feeling of a cool forehead.[29] This method is beneficial because once trained in these procedures, an individual can carry out this treatment independently.[30]

Autogenic training could then be performed at home, which could increase adherence to a training schedule. Research evaluating the autogenic training relaxation technique has resulted in empirical support for several outcomes including but not limited to reducing anxiety,[30,31,32] and enhancing stress-management well-being in participants.[33] Findings through systematic review performed by Ernst and Kanji (2000) also found autogenic training to relieve stress in seven out of eight cases, and findings by Lim & Kim (2014) have shown autogenic training to be a valuable approach to reducing stress in nursing students, a college student population known for having increased stress, according to Lim & Kim. Its specific use to treat stress-aggravated diseases such as irritable bowel syndrome,[34] cancer,[35] and multiple sclerosis[36] suggests autogenic training may be effective for individuals diagnosed with T1DM. This result was supported by Murakami and colleagues' (2006) findings that autogenic training shows improvement of endocrine function.

With evidence that autogenic training could potentially be useful in both the stress reduction and glycemic control of T1DM, the authors created the following study in an attempt to test the following hypothesis. The purpose of the present study was to evaluate the effect of autogenic training on college students diagnosed with type 1 diabetes mellitus on self-reported stress and glycemic control. The autogenic training was evaluated using a multiple baseline design with the participant. Reduction in stress was measured with a

29 Luthe, W., Schultz, J. H. 1969. *Autogenic Therapy*. Vol. 1. New York: Grune & Stratton.
30 Kanji, N., White, A., & Ernst, E. 2006. Autogenic training to reduce anxiety in nursing students: randomized controlled trial. *Journal of Advanced Nursing, 53*(6): 729–735.
31 Holland, B., Gosselin, K., & Mulcahy, A. 2017. The effect of autogenic training on self- efficacy, anxiety, and performance on nursing student simulation. *Nursing Education Perspectives, 38*(2): 87–89.
32 Murakami, M., Koike, K., Ashihara, M., Matsuno, T., Tazoe, M., & Katsura, T. 2006. Recent advance of autogenic training in clinical practice of psychosomatic medicine in Japan. *International Congress Series, 1287:* 240–245.
33 Yurdakul, L., Holttum, S., & Bowden, A. 2009. Perceived changes associated with autogenic training for anxiety: a grounded theory study. Psychological and Psychotherapy: *Theory, Research and Practice, 82*(4): 403–419.
34 Shinozaki, M., Kanazawa, M., Kano, M., Endo, Y., Nakaya, N., Hongo, M., & Fukudo, S. 2010. Effect of autogenic training on general improvement in patients with irritable bowel syndrome: a randomized controlled trial. *Applied Psychophysiology and Biofeedback, 35*(3): 189–198.
35 Wright, S., Courtney, U., & Crowther, D. 2002. A quantitative and qualitative pilot study of the perceived benefits of autogenic training for a group of people with cancer. *European Journal of Cancer Care, 11*(2): 122–130.
36 Sutherland, G., Andersen, M. B, & Morris, T. 2005. Relaxation and health-related quality of life in multiple sclerosis: the example of autogenic training. *Journal of Behavioral Medicine, 28*(3): 249–256.

variety of psychological measures, and improvement of glycemic control was measured with daily blood glucose levels. We hypothesized, by introducing daily autogenic training into the schedule of a college student with T1DM, we could both reduce their stress and improve their glycemic control.

Method

Participants

The single participant in this case study was an 18-year-old student from an undergraduate psychology course at a Midwestern university. The participant had been diagnosed with type 1 diabetes prior to the study. Recruitment for this study was conducted online following approval from the university's institutional review board by using the university's e-mail system.

Apparatus

The participant recorded all consent, data, and survey responses via the Qualtrics survey software. This software was made accessible via an anonymous link distributed to the participant via the researchers. All surveys were issued at the beginning and end of the baseline period and at the end of the intervention period of the study. Blood glucose measures were also recorded via a separate anonymous link sent to the participant using the Qualtrics system.

The autogenic training implemented in the intervention phase of this study was issued via an MP3 file download sent to the participant. The MP3 file contained the autogenic training audio file labeled "Shealy Mini Basic Biogenics," which is a seven-minute guide created by Dr. Norman Shealy (2018) as a shorter adaptation from J.H. Schultz's (1932) original autogenic training audio file of 18 minutes.

An example of this audio instruction is as follows: "As you breathe in, say to yourself, 'my arms and legs,' and as you breathe out, 'are heavy and warm … my arms and legs are heavy and warm.'" The audio contains "commands," which teach the listener's body to relax and control their breathing, which according to Schultz, is thought to be the reason for the stress reduction.

Materials

The physiological measure used to analyze glycemic control was blood glucose, which was measured two times daily: once in the morning and once in the afternoon during the baseline and intervention phases. The authors collected data twice daily because research by Calisti and Tognetti suggests

when afternoon and morning blood glucose levels are compared, they better correlate to HbA1c levels.[37] Blood glucose readings are a form of instantaneous blood glucose level measure that allow the researchers to detect a daily change in glycemic control.[38]

Six psychological measures were used during this study at both the beginning and end of the intervention phase. The first is the Diabetes Distress Scale (DDS), which has shown Cronbach's alpha of 0.92.[39] Chronbach's alpha is a measure of internal consistency. The DDS uses a five-point Likert scale that is then transformed to a 0–100 scale to measure both diabetes-related problem areas and emotional distress. A score of 100 indicates greater distress. There are four subscales in this measure that will be evaluated: emotional burden, physician-related distress, regimen distress (stress related to the patient's medical regimen), and diabetes-related interpersonal distress.

The Depression Anxiety Stress Scale (DASS) is a measure that uses a four-point Likert scale from 0 ("did not apply to me at all") to 3 ("applied to me very much, or most of the time") to measure depression, anxiety, and stress.[40] Higher scores indicate higher levels of depression, anxiety, or stress. These measures demonstrate a Cronbach's alpha for each of these areas of 0.94, 0.87, and 0.91 respectively.[41]

The Problem Areas in Diabetes (PAID) scale uses a six-point Likert scale[42] that is transformed to a 0–100 scale, with 100 indicating greater distress, and is used to measure psychosocial adjustment specific to diabetes. This scale has shown to be reliable with a Cronbach's alpha of 0.95.

The Rand-36 scale was used to measure the health-related quality of life (HRQoL) of the participant.[43] All health concepts the scale assesses are transformed into a 0–100 scale and then averaged. A variety of subscales are found within the RAND-36 scale as present in *Table 1*. This item has shown reliability with a Cronbach's alpha of 0.90. This scale was used because it allowed the researchers to determine the impact of the participant's health on the participant's perception and ability to function in the physical, mental, and social domains of life.

37 Calisti, L., & Tognetti, S. 2005. Measure of glycosylated hemoglobin. *Acta Bio-Medica: Atenei Parmensis, 76*(3): 59–62.
38 Bunn, H. F. 1981. Evaluation of glycosylated hemoglobin in diabetic patients. *Diabetes, 30*: 613–617.
39 Graue, M., Haugstvedt, A., Wentzel-Larsen, T., Iversen, M. M., Karlsen, B., & Rokne, B. 2012. Diabetes-related emotional distress in adults: reliability and validity of the Norwegian versions of the Problem Areas in Diabetes scale (PAID) and the Diabetes Distress Scale (DDS). I*nternational Journal of Nursing Studies, 49*: 174–182.
40 Antony, M. M., Cox, B. J., Enns, M. W, Bieling, P. J., & Swinson, R. P. 1998. Psychometric properties of the 42-item and 21-item versions of the depression anxiety stress scales in clinical groups and a community sample. *Psychological Assessment, 10*(2): 176–181.
41 Gosling, S. D., Rentfrow, P. J., & Swann, W. B. 2003. A very brief measure of the big-five personality domains. *Journal of Research in Personality, 37*: 504–528.
42 Polonsky, W. H., Anderson, B. J., Lohrer, P. A., Welch, G., Jacobson, A. M., Aponte, J. E., & Schwartz, C. E. 1995. Assessment of diabetes-related distress. *Diabetes Care, 18*(6): 754–760.
43 Hays, R. D., & Moralesm L. S. 2001. The RAND-36 measure of health-related quality of life. *Annals of Medicine, 33*: 350–357.

The researchers used the Ten-Item-Personality-Inventory (TIPI) to measure the five personalities of extroversion, agreeableness, conscientiousness, emotional stability, and openness.[41] It uses a seven-point scale: A higher score indicates greater presence of that personality trait. This measure has shown a respective Cronbach's alpha of 0.68, 0.40, 0.50, 0.73, and 0.45 for the different personalities. Although the measurement of reliability is below excellent due to its adaption for use during a limited time, the TIPI measure has demonstrated adequacy in terms of convergence with the more established Big-Five measure, test-retest reliability, and external correlation patterns. Researchers used the TIPI to find established correlations between personality, stress, and/or diabetic glycemic control and aim to determine if personality measures could suggest stress.

The final psychological measure used is the Coping Competence Questionnaire (CCQ). This measure has demonstrated a Cronbach's alpha between 0.87 and 0.94.[44] It uses a six-point Likert scale that sums and reverses scores; higher scores indicate higher coping competence. This measure indicates coping competence, a stress resistance factor in the participants that indicates the individual's ability to effectively cope with failure and/or negative life events and will be used by the researchers to determine changes in coping competence after the use of autogenic training.

Procedure

After the participant was recruited and researchers obtained her consent, researchers held an informational meeting with her to cover the details, duration, and schedule of the study. This meeting marked the beginning of the 19-day baseline period. The participant then took the six psychological measure questionnaires and began recording blood glucose levels twice a day—once in the morning and once in the afternoon. She submitted blood glucose measures via the Qualtrics system each Sunday through the 19-day baseline period and 55-day intervention period. The mean of the two daily readings provided a daily average blood glucose measure, which was used to detect changes in blood glucose levels throughout the study.

After the 19-day baseline period, the participant attended another meeting with a researcher. At this meeting, the participant completed the six psychological measures via Qualtrics. Additionally, the researcher provided a demonstration of the autogenic training procedure using the audio file. The participant was instructed to complete the relaxation training activity at least once a day during the intervention phase. This began the eight-week intervention

44 Schroder, K. E. E. 2004. Coping competence as predictor and moderator of depression among chronic disease patients. *Journal of Behavioral Medicine, 27* (2): 123–145.

period, which complied with the recommendation to conduct the autogenic training technique over an eight to 10-week period.[45]

The autogenic training was re-demonstrated at weeks three and five of the intervention phase to maintain the participant's implementation fidelity. At the end of the 10-week experimental period, the participant attended a final meeting where she completed the six posttest psychological measures via the Qualtrics survey software. The final form to submit weekly blood glucose levels was sent to the participant the Sunday before the meeting.

Daily blood glucose levels were plotted and evaluated using visual analysis. An average baseline blood glucose level was found and the blood glucose levels from the 19-day intervention phase were averaged so a final intervention blood glucose average could be found. Scores from the psychological questionnaires were evaluated for changes between the pre-baseline, intervention onset, and post-intervention scorings.

The current study was a case study experiment. The independent variable was the daily use of the autogenic training breathing technique by the participant, and the dependent variable was self-indicated stress and blood glucose levels.

Results

Autogenic training was evaluated to determine if the intervention was effective in improving glycemic control and reducing self-indicated stress. As hypothesized, the blood glucose average for the participant was reduced from 207.5 mg/dL during the baseline phase to 185.52 mg/dL during the intervention phase. The participant's final 19 days of intervention was used as a comparison to the 19-day baseline period. The final 19 days of intervention demonstrated an average blood glucose of 176.92 mg/dL (*see Figure 2*). Although there was not a clear trend in the data during either phase, 65.45% of the blood glucose daily averages during the intervention phase were below the baseline mean (*see Figure 1*). Also during the intervention phase, seven data points occurred within the non-diabetic range of 68–126 mg/dL, compared to 0 data points during the baseline phase.

The participant's self-reported changes in psychological well-being as measured by the six self-reported questionnaires (*see Table 1*) did not reliably demonstrate improvement from baseline reports. All areas of the TIPI showed little change over the course of the study. The Rand-36 Health and Wellness questionnaire showed no change at any point in the study for physical functioning, role limitation due to physical health, or health change. Role limita-

45 Therapy Directory. Autogenic Training. 2018.

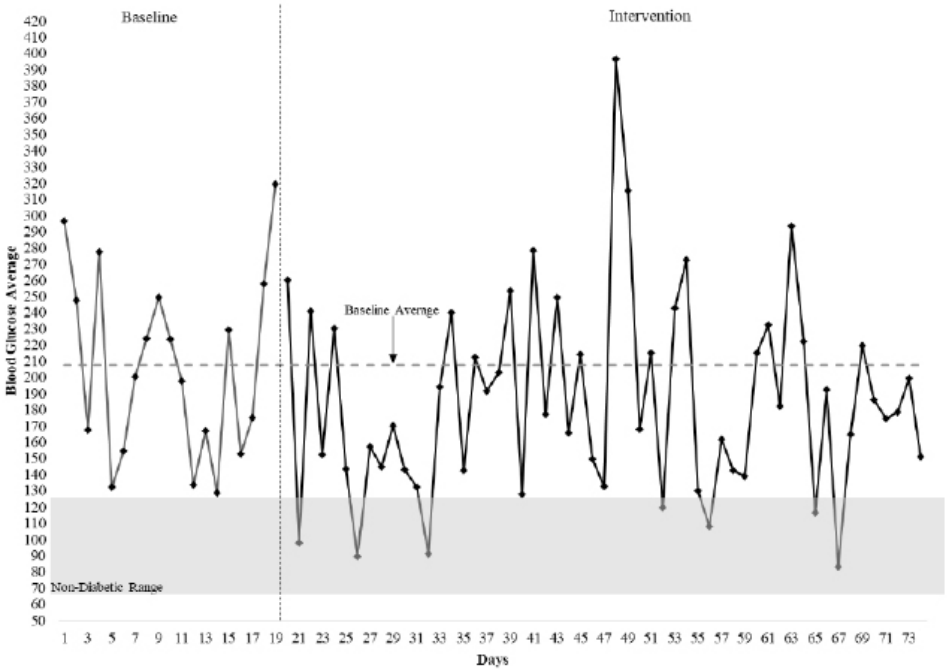


Figure 1: Blood glucose daily average during baseline and intervention conditions. Mean baseline average is identified with a horizontal dashed line, and the light grey box indicates blood glucose levels in the non-diabetic range (68–126 mg/dL).

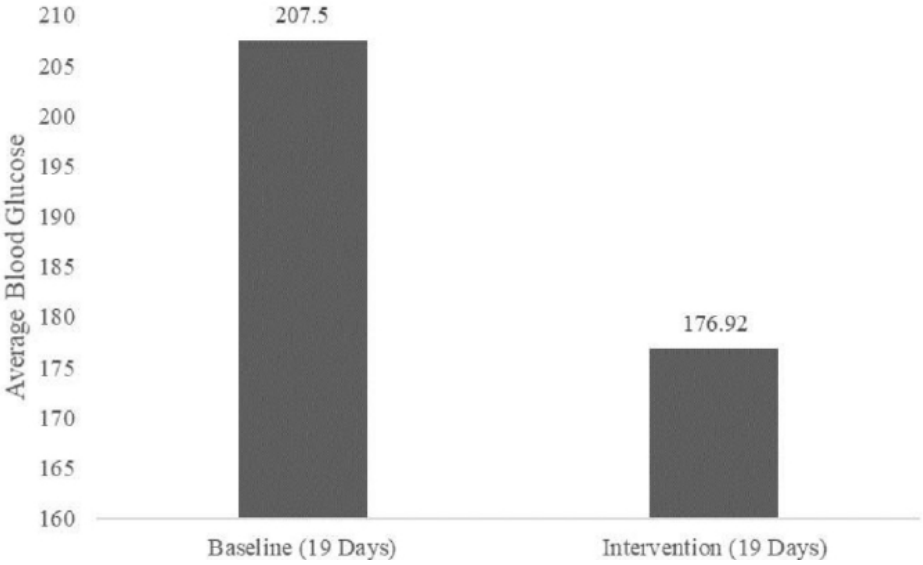


Figure 2: Mean blood glucose reports during baseline (19 days) and final 19 days of intervention.

Table 1. Psychological Self-Report Measures			
	Pre-Baseline	**Intervention Onset**	**Post-Intervention**
Ten Items Personality Inventory (maximum score 7) *			
Extroversion	2	4	2
Agreeableness	5	5	5.5
Conscientiousness	7	7	7
Emotional Stability	2	3	1.5
Openness	6	5.5	5.5
RAND-36 (Health and Wellness; maximum score 100) *			
Physical Functioning	100	100	100
Role Limit Physical	100	100	100
Role Limit Emotional	67	67	0
Energy Fatigue	25	20	30
Emotional Well-being	52	56	40
Social Functioning	63	75	88
Pain	80	80	100
General Health	80	75	85
Health Change	50	50	50
Coping Competence Questionnaire (maximum score 6) *			
	3.75	3.75	2.67
Depression, Anxiety, and Stress Scale (maximum score 3) **			
Depression	0.57	0.29	1.57
Anxiety	0.14	0.43	1.57
Stress	1.14	1.43	2.57
**Problem Areas in Diabetes Scale (maximum score 100) **			
	40	33.75	37.5
**Diabetes Distress Scale (maximum score 100) **			
Emotional Burden	75	85	70
Physician-Related Distress	0	0	0
Regimen Distress	65	65	30
Interpersonal Distress	33.33	16.67	8.33
Summary	43.33	41.67	27.08
** higher scores on this measure indicate improvement*			
*** lower scores on this measure indicate improvement*			

tions due to emotional problems changed from 67 during both pre-intervention measures to 0 post-intervention. Other subscales noted lesser changes: Energy fatigue changed from 25 pre-baseline to 20 at intervention onset to 30 in post-intervention measures, emotional well-being changed from 52 to 56 to 40, social functioning changed from 63 to 75 to 88, general health changed from 80 to 75 to 85, and pain changed from 80 during both pre-intervention tests to 100 post intervention. On the Rand-36 scale, an increased score indicated improvement. The CCQ changed from 3.75 during other pre-intervention tests to 2.67 post-intervention indicating a decline in coping competence. All measures of the DAS scale showed a decline in improvement: Depression changed from 0.57 to 0.29 to 1.57, anxiety changed from 0.14 to 0.43 to 1.57, and stress changed from 1.14 to 1.43 to 2.57. The PAID scale also showed inconclusive results with scores changing from 40 to 33.75 to 37.5. A lower score on the PAID scale indicates improvement. The only measure that produced improved scores on all subtests was the Diabetes Distress Scale. In this scale emotional burden changed from 75 to 85 to 70, physician-related distress remained at 0 during all tests, regimen distress changed from 65 on both pre-intervention measures to 30 post-intervention, and interpersonal distress changed from 33.3 to 16.67 to 8.33. The overall score for the DDS measure changed from 43.33 at pre-baseline to 41.67 at intervention onset and then to 27.08 post-intervention. A decrease in score indicated an improvement for the DDS.

Discussion

The participant's mean blood glucose was 207.05 mg/dL in baseline (19 days) and 176.92 mg/dL at the end of the intervention (final 19 days). This demonstrates a likely A1C (a common measure of blood glucose)[46] change from 8.8–7.8% As noted by the Centers for Disease Control (2011), a 1% drop generally indicates a 40% reduced risk of microvascular complications such as eye, kidney, and nerve diseases. During the intervention period, the participant had seven blood glucose measurements within the non-diabetic range of 68–126 mg/dL:[47] This did not occur in the baseline period. These points show the participant was able to not only reduce her blood glucose mean but also maintain a tighter glycemic control near the non-diabetic blood glucose range.

The participant's reports on psychological measures did not indicate an improvement in most areas. However, the participant's reports on the Diabe-

46 Nathan, D. M., Kuenen, J., Borg, R., Zheng, H., Schoenfeld, D., & Heine, R. J. 2008. Translating the A1C assay into estimated average glucose levels. *Diabetes Care, 31*(8): 1473–1478.

47 Sutherland, G., Andersen, M. B, & Morris, T. 2005. Relaxation and health-related quality of life in multiple sclerosis: the example of autogenic training. *Journal of Behavioral Medicine, 28*(3): 249–256.

tes Distress Scale did suggest all areas of distress related to diabetes decreased. This could indicate the autogenic training did reduce diabetes-related stress but had a lesser effect on other stressors in the participant's life. It is possible the participant's knowledge of lower blood glucose readings during the intervention phase affected her responses on the questionnaire. This knowledge could be due to the participant understanding the goal of autogenic training was to improve glycemic control and reduce stress, which caused her to respond in a way that indicated improvement in glycemic control and reduction in stress related to diabetes.

As stated in previous research, psychological distress is prevalent among diabetics in the United States.[48] This current study was expected to demonstrate the participant's indicated distress associated with their diagnosis of diabetes. Although the participant's diabetes-related distress did decrease from baseline measure to post-intervention measures, the participant did not show abnormally high levels of diabetes-related stress in the baseline. Indicators of stress were also expected to be found in the study's participant in agreement with Nigro (1981), who stated stress is a major problem in the student population. Diabetes-related stress was also not evident in the beginning of the study, although higher levels of stress were found post-intervention. This increase in the participant's stress was expected to present with worsened glycemic control, but since glycemic control was found to improve, results could imply glycemic control is more closely related to diabetes-related distress. Although this is just one potential implication of the data, future research could assess the relationship between glycemic control and diabetes-related distresses by evaluating various effects of diabetes-related and non-diabetes-related stressors. Replications of the current study on a larger scale may provide more evidence for the use of autogenic training as a glycemic control method for patients with T1DM.

With increases in both stress and anxiety after the intervention of autogenic training, our results were not found to coincide with the findings of Kanji, White, and Ernst (2006). These findings may be due to other stressors unrelated to the study since the study was not performed in a controlled setting. However, the reduction in diabetes-related distress and improvement of glycemic control found in this college student with type 1 diabetes from autogenic training shows a potential for its use with type 1 diabetics in the future. This study may indicate autogenic training can be used to treat type 1 diabetes

48 Li, C., Ford, E. S., Zhao, G., Strine, T. W, Dhingra, S., Barker, L., ... Mokdad, A. H. 2009. Association between diagnosed diabetes and serious psychological distress among U.S. adults: the Behavioral Risk Factor Surveillance System, 2007. *International Journal of Public Health, 54* (Suppl 1): 43–51.

similarly to its use in treating other diseases aggravated by distress.[49,50,51] As a way to reduce diabetes-related stress and improve physiological glycemic control, our results for the use of autogenic training in college students with type 1 diabetes shows promise.

A limitation of this study was the participant's self-monitoring of blood glucose levels were not observed, although this was part of her existing diabetes care regimen. Additionally, blood glucose levels seemed to rise during the participant's spring break, which may relate to irregular activity and could have affected other components of the participant's diabetic care regimen (such as diet and exercise), which were not measured during this study. Finally, the number of stressors present in the participant's environment may have fluctuated during the course of the study, which may have influenced the amount of autogenic training necessary to address physiological effects of D1TM.

The single subject sample limits this study's representation of the general population. With the sample size of this study as a limitation, it is suggested a larger sample of college students with type 1 diabetes be used in future studies to obtain more significant results and indicate a larger effect. Attrition, when participants leave a study, is another factor that could lead to a smaller sample size and smaller effect, which increases the need for future studies with a larger sample size. Because it is known that college students have higher stress levels compared to other age groups, generalization to other populations may be difficult. Therefore it is suggested that studies be performed on larger samples with both middle-aged and older adult populations as well as child and adolescent populations to see if similar data results. Additional studies could extend the use of autogenic training beyond the college student population with type 1 diabetes.

The results found by this case study, despite limitations, are promising. With future research, the use of autogenic training as a method to reduce distress and improve glycemic control in individuals with T1DM may yield further positive results. The major recommendation for future research is to replicate with a larger, more diverse sample for a longer period. Larger samples may help alleviate any confounds related to changes in environmental stressors for the present case study. Future studies examining a combination of autogenic training with another diabetic therapy technique or techniques,

49 Shinozaki, M., Kanazawa, M., Kano, M., Endo, Y., Nakaya, N., Hongo, M., & Fukudo, S. 2010. Effect of autogenic training on general improvement in patients with irritable bowel syndrome: a randomized controlled trial. *Applied Psychophysiology and Biofeedback, 35*(3): 189–198.
50 Wright, S., Courtney, U., & Crowther, D. 2002. A quantitative and qualitative pilot study of the perceived benefits of autogenic training for a group of people with cancer. *European Journal of Cancer Care, 11*(2): 122–130.
51 Sutherland, G., Andersen, M. B., & Morris, T. 2005. Relaxation and health-related quality of life in multiple sclerosis: the example of autogenic training. *Journal of Behavioral Medicine, 28*(3): 249–256.

in order to reduce physiological distress for greater improvement in glycemic control, are also recommended as a future direction for this research.

References

Amsberg, S., Anderbro, T., Wredling, R., Lisspers, J., Lins, P., Adamson, U., & Johansson, U. (2009). A cognitive behavior therapy-based intervention among poorly controlled adult type 1 diabetes patients — a randomized controlled trial. *Patient Education and Counseling, 77*(1): 72–80. https://doi.org/10.1016/j.pec.2009.01.015

Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2001). The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care, 24*(6): 1069–1078. https://doi.org/10.2337/diacare.24.6.1069

Antony, M. M., Cox, B. J., Enns, M. W., Bieling, P. J., & Swinson, R. P. (1998). Psychometric properties of the 42-item and 21-item versions of the depression anxiety stress scales in clinical groups and a community sample. *Psychological Assessment, 10*(2): 176–181. https://doi.org/10.1037/1040-3590.10.2.176

Bunn, H. F. (1981). Evaluation of glycosylated hemoglobin in diabetic patients. *Diabetes, 30*: 613–617. https://doi.org/10.2337/diab.30.7.613

Calisti, L., Tognetti, S. (2005). Measure of glycosylated hemoglobin. *Acta Bio-Medica: Atenei Parmensis, 76*(3): 59–62. PMID: 16915800

Centers for Disease Control and Prevention. (2011). National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.cdc.gov/diabetes/pubs/pdf/methods11.pdf

Chang, S. B., Kim, S. Y., Oh, S. K., Yoo, I. Y., Kim, H. S., Kim, S., ... Choi, E. K. (2007). The effects of guided imagery program on high school male students' stress, stress coping, and health problems. *Journal of Korean Academy of Psychiatric and Mental Health Nursing, 16*(1): 41–50. https://www.kci.go.kr/kciportal/ci/sereArticleSearch/ciSereArtiView.kci?sereArticleSearchBean.artiId=ART001047990

Cohen, A. S., Vance, V. K., Runyan, J. W., & Hurwitz, D. (1960). Diabetic acidosis: an evaluation of the cause, course, and therapy of 73 cases.

Annals of Internal Medicine,52(1): 55–86. https://doi.org/10.7326/0003-4819-52-1-55

Cox, D. J., Taylor, A. G., Nowacek, G., Holley-Wilcox, P., Pohl, S. L., & Guthrow, E. (1984). The relationship between psychological stress and insulin-dependent diabetic blood glucose control: preliminary investigations. *Health Psychology, 3*(1): 63–75. https://doi.org/10.1037/0278-6133.3.1.63

Devries, J. H., Snoek, F. J., & Heine, R. J. (2004). Persistent poor glycemic control in adult type 1 diabetes. A closer look at the problem. *Diabetic Medicine 21*(12): 1263–1268. https://doi.org/10.1111/j.1464-5491.2004.01386.x

Diabetes Control and Complications Trial Research Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England Journal of Medicine, 329*: 977–986. https://doi.org/10.1056/NEJM199309303291401

Diabetes Control and Complications Trial Research Group. (1997). Hypoglycemia in the diabetes control and complications trial. *Diabetes, 46*: 271–286. PMID: 9000705

Ernst, E., & Kanji, N. (2000). Autogenic training for stress and anxiety: a systematic review. *Complementary Therapies in Medicine, 8*(2): 106–110. https://doi.org/10.1054/ctim.2000.0354

Folkman, S., & Lazarus, R. S. (1980). An analysis of coping in a middle-aged community sample. *Journal of Health and Social Behavior, 21*(3): 219–239. https://doi.org/10.2307/2136617

Fowler, M. J. (2008). Microvascular and macrovascular complications of diabetes. *Clinical Diabetes, 26*(2): 77–82. https://doi.org/10.2337/diaclin.26.2.77

Geddes, J., McGeough, E., & Frier, B. M. (2006). Young adults with type 1 diabetes in tertiary education: do students receive adequate specialist care?

Diabetic Medicine, 23(10): 1155–1157. https://doi.org/10.1111/j.1464-5491.2006.01933.x

Gosling, S. D., Rentfrow, P. J., & Swann, W. B. (2003). A very brief measure of the big-five personality domains. *Journal of Research in Personality, 37*: 504–528. https://doi.org/10.1016/S0092-6566(03)00046-1

Graue, M., Haugstvedt, A., Wentzel-Larsen, T., Iversen, M. M., Karlsen, B., & Rokne, B. (2012). Diabetes-related emotional distress in adults: reliability and validity of the Norwegian versions of the Problem Areas in Diabetes scale (PAID) and the Diabetes Distress Scale (DDS). *International Journal of Nursing Studies, 49*: 174–182. https://doi.org/10.1016/j.ijnurstu.2011.08.007

Grigsby, A. B., Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2002). Prevalence of anxiety in adults with diabetes: a systematic review. *Journal of Psychosomatic Research, 53*(6): 1053–1060. https://doi.org/10.1016/S0022-3999(02)00417-8

Hays, R. D., & Morales, L. S. (2001). The RAND-36 measure of health-related quality of life. *Annals of Medicine, 33*: 350–357. https://doi.org/10.3109/07853890109002089

Hislop, A. L., Fegan, P. G., Schlaeppi, M. J., Duck, M., & Yeap, B. B. (2008). Prevalence and associations of psychological distress in young adults with type 1 diabetes. *Diabetic Medicine, 25*: 91–96. https://doi.org/10.1111/j.1464-5491.2007.02310.x

Holland, B., Gosselin, K., & Mulcahy, A. (2017). The effect of autogenic training on self-efficacy, anxiety, and performance on nursing student simulation. *Nursing Education Perspectives, 38*(2): 87–89. https://doi.org/10.1097/01.NEP.0000000000000110

Ismail, K., Thomas, S. M., Maissi, E., Chalder, T., Schmidt, U., Bartlett, J., ... Treasure, J. (2008). Motivational enhancement therapy with and without cognitive behavioral therapy to treat type 1 diabetes patients: a randomized trial. *Annals of Internal Medicine, 149*(10): 708–719. https://doi.org/10.7326/0003-4819-149-10-200811180-00005

Jevning, R., Wilson, A. F., & Davidson, J. M. (1978). *Adrenocortical activity*

during meditation. Hormones and Behavior, 10(1): 54–60. https://doi.org/10.1016/0018-506X(78)90024-7

Kanji, N., White, A., & Ernst, E. (2006). Autogenic training to reduce anxiety in nursing students: randomized controlled trial. *Journal of Advanced Nursing, 53*(6): 729–735. https://doi.org/10.1111/j.1365-2648.2006.03779.x

Li, C., Ford, E. S., Zhao, G., Strine, T. W., Dhingra, S., Barker, L., ... Mokdad, A. H. (2009). Association between diagnosed diabetes and serious psychological distress among U.S. adults: The Behavioral Risk Factor Surveillance System, 2007. *International Journal of Public Health, 54* (Suppl 1): 43–51. https://doi.org/10.1007/s00038-009-0006-1

Lim, S., & Kim, C. (2014). Effects of autogenic training on stress response and heart rate variability in nursing students. *Asian Nursing Research, 8*: 286–292. https://doi.org/10.1016/j.anr.2014.06.003

Lloyd, C. E., Dyer, P. H., Lancashire, R. J., Harris, T., Daniels, J. E., & Barnett, A. H. (1999). Association between stress and glycemic control in adults with type 1 (insulin-dependent) diabetes. *Diabetes Care, 22*(8): 1278–1283. https://doi.org/10.2337/diacare.22.8.1278

Luthe, W., & Schultz, J. H. (1969). *Autogenic Therapy*. Vol. 1. New York: Grune & Stratton.

McCrimmon, R. J., Ryan, C. M., & Frier, B. M. (2012). Diabetes and cognitive dysfunction. *Lancet, 379*: 2291–2299. https://doi.org/10.1016/S0140-6736(12)60360-2

Miller, W. R., Zweben, A., DiClemente, C. C., & Rychtarik, R. G. (1994). Motivational enhancement therapy manual: A clinical research guide for therapists treating individuals with alcohol abuse and dependence. Rockville, MD: NIAAA. https://permanent.access.gpo.gov/gpo37581/match02.pdf

Nabarro, J. D. N. (1965). Diabetic acidosis: clinical aspects. *On the nature and treatment of diabetes*, 545–557.

Murakami, M., Koike, K., Ashihara, M., Matsuno, T., Tazoe, M., & Katsu-

ra, T. (2006). Recent advance of autogenic training in clinical practice of psychosomatic medicine in Japan. *International Congress Series, 1287*: 240–245. https://doi.org/10.1016/j.ics.2005.12.054

Nathan, D. M., Kuenen, J., Borg, R., Zheng, H., Schoenfeld, D., & Heine, R. J. (2008). Translating the A1C assay into estimated average glucose levels. *Diabetes Care, 31*(8): 1473–1478. https://doi.org/10.2337/dc08-0545

Nigro, A. G. (1981). Struggling with stress. J*ournal of College Student Personnel, 22*: 373.

Noordali, F., Cumming, J., & Thompson, J. L. (2017). Effectiveness of mindfulness-based interventions on physiological and psychological complications in adults with diabetes: a systematic review. *Journal of Health Psychology, 22*(8): 965–983. https://doi.org/10.1177/1359105315620293

Pelletier, K. R. (1979). "Holistic medicine: from pathology to prevention." *The Western Journal of Medicine, 131*(6): 481–483. https://www.ncbi.nlm.nih.gov/pmc/issues/123619/

Plack, K., Herpertz, S., & Petrak, F. (2010). Behavioral medicine interventions in diabetes. *Current Opinion in Psychiatry, 23*: 131–138. https://doi.org/10.1097/YCO.0b013e3283366555

Polonsky, W. H., Anderson, B. J., Lohrer, P. A., Welch, G., Jacobson, A. M., Aponte, J. E., & Schwartz, C. E. (1995). Assessment of diabetes-related distress. *Diabetes Care, 18*(6): 754–760. https://doi.org/10.2337/diacare.18.6.754

Porte, D., & Schwartz, M. W. (1996). Diabetes complications—Why is glucose potentially toxic? *Science, 272* (5262): 699. https://www.jstor.org/stable/2889437

Schabert, J., Brown, L., Mosely, K., & Speight, J. (2013). Social stigma in diabetes: A framework to understand a growing problem for an increasing epidemic. *The Patient: Patient-Centered Outcomes Research, 6*(1): 1–10. https://doi.org/10.1007/s40271-012-0001-0

Schroder, K. E. E. (2004). Coping competence as predictor and moderator of

depression among chronic disease patients. J*ournal of Behavioral Medicine, 27* (2): 123–145.https://doi.org/10.1023/B:JOBM.0000019848.84779.a9

Schultz, J. H. (Presenter/Producer). (1932). Basic Schultz – Autogenic Training [Audio file]. http://www.mediafire.com/file/tk1qfax2hl22hbv/Basic+-Schultz.mp3

Shealy, N. (Producer). (2018). Shealy Mini Basic Biogenics. Retrieved from audio file.

Shinozaki, M., Kanazawa, M., Kano, M., Endo, Y., Nakaya, N., Hongo, M., & Fukudo, S. (2010). Effect of autogenic training on general improvement in patients with irritable bowel syndrome: a randomized controlled trial. *Applied Psychophysiology and Biofeedback, 35*(3): 189–198. https://doi.org/10.1007/s10484-009-9125-y

Surwit, R. S., Scovern, A. W., & Feinglos, M. N. (1982). The role of behavior in diabetes care. *Diabetes Care, 5*: 337–342. https://doi.org/10.2337/diacare.5.3.337

Surwit, R. S., van Tilburg, M. A., Zucker, N., McCaskill, C. C., Parekh, P., Feinglos, M. N., ... P., Lane, J. D. (2002). Stress management improves long- term glycemic control in type 2 diabetes. *Diabetes Care, 25*(1): 30–34. https://doi.org/10.2337/diacare.25.1.30

Sutherland, G., Andersen, M. B., & Morris, T. (2005). Relaxation and health-related quality of life in multiple sclerosis: the example of autogenic training. *Journal of Behavioral Medicine, 28*(3): 249–256. https://doi.org/10.1007/s10865-005-4661-2

Therapy Directory. Autogenic Training. (2018). https://www.therapy-directory.org.uk/articles/autogenic-training.html

World Health Organization. (2021). Fact sheets: diabetes https://www.who.int/news-room/fact-sheets/detail/diabetes

Wright, S., Courtney, U., & Crowther, D. (2002). A quantitative and qualitative pilot study of the perceived benefits of autogenic training for a group of people with cancer. *European Journal of Cancer Care, 11*(2):

122–130. https://doi.org/10.1046/j.1365-2354.2002.00307.x

Yurdakul, L., Holttum, S., & Bowden, A. (2009). Perceived changes associated with autogenic training for anxiety: a grounded theory study. *Psychology and Psychotherapy: Theory, Research and Practice, 82*(4): 403–419. https://doi.org/10.1348/147608309X444749

Angiogenesis in Microvascular Endothelial Cells Stimulated by UTP: P2Y$_2$ Receptor and Vascular Endothelial Growth Factor Receptor 2

Leanna Zelle, Abby Gann, and Jianjie Wang, MD, PhD

Abstract

Extracellular ATP and UTP act as signaling molecules and equally activate the nucleotide P2Y$_2$ receptor (P2Y$_2$R). Angiogenesis mediated by P2Y$_2$R is not well characterized in microvascular endothelial cells (MEC). We hypothesized P2Y2R would promote angiogenesis via Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), an integral membrane marker protein in proliferating and angiogenic MEC. We used MEC cells isolated from wild-type (WT) and P2Y$_2$R knockout (KO) mice to test the hypothesis. VEGFR-2 expression level in WT-MEC and KO-MEC was assessed by using flow cytometry assay under resting and UTP stimulation conditions over time. The angiogenic function of WT-MEC was assessed by using capillary-like tube formation assay under resting and UTP stimulation conditions over time. We found baseline VEGFR-2 expression was lower in P2Y$_2$R KO-MEC compared with WT-MEC. P2Y$_2$R activation by UTP altered VEGFR-2 expression and promoted capillary-like tube formation in WT-MEC. However, UTP stimulation did not alter VEGFR-2 expression level compared with the baseline in P2Y$_2$R KO-MEC. These findings indicate that P2Y$_2$R activation alters VEGFR-2 expression and stimulates angiogenesis in MEC. In the future studies, we will further test if VEGFR-2 mediates P2Y$_2$R-dependent angiogenesis.

Introduction

Microvascular Angiogenesis

Of the vast vascular network in the human body, microvasculature makes up a majority of the total length and surface area, allowing for ample blood-tissue exchange and micro-regulation as the barrier between the tissue and the blood. Microvasculature is unique compared to large vasculature because the arrangement and structure is precisely regulated by an array of stimuli from the local environment, whereas the location of large vasculature is relatively fixed. Local chemical or mechanical environmental stimuli can lead to the regulation of vasculogenesis, angiogenesis, intussusception, remodeling, maturation, and pruning of the microvessels (Secomb & Pries, 2016). These processes are often studied as independent points of regulation but may be better understood if studied as a continuous, integrated system. However, to best study all aspects of vasculature regulation simultaneously, a full baseline understanding of each process is necessary. In this study, the focus is on angiogenesis in the context of $P2Y_2R$ and VEGFR-2.

The process of angiogenesis occurs under extensive crosstalk, with 240 proteins identified for their involvement with VEGFR-2 mediated signal transduction events. The initiation of angiogenesis requires the activation of endothelial cells, which leads to specialized phenotypes (Abhinand et. al, 2016). One phenotype, the activated tip endothelial cells, use their abundant filopodia to migrate to areas of low vascularization while working with the stalk endothelial cell phenotype that functions to provide stability for vessel sprouting (Abhinand et. al, 2016; Motherwell et. al, 2018). Angiogenesis is just one piece of the complex regulation of microvasculature, but is important for the health and survival of mammalian organisms as it plays a role in many vital physiological processes; thus angiogenic dysfunction is present in various local and systemic pathologies.

Understanding angiogenesis is vital in the treatment of many pathologies. For example, down regulation angiogenesis is a target for potentially slowing or inhibiting tumor growth (Secomb & Pries, 2016). CRISPR-Cas 9, for example, has been used to target VEGFR-2 and downregulate microvascular angiogenesis *in vitro* as a potential treatment to prevent cancer vascularization (Wu et. al, 2017). On the other hand, upregulation of angiogenesis has been studied in order to aid pathologies like diabetic wound healing, where the VEGF ligand can enhance local and systemic wound healing by upregulating angiogenic and proliferative factors such as VEGFR-2 (Galiano et. al, 2004). In the context of myocardial infarction, microvascular angiogenesis is import-

ant for a successful recovery (Motherwell et. al, 2018). Whether the endothelial cells are targeted to upregulate or downregulate angiogenic activities, understanding the complex process of angiogenesis in great detail is essential if these therapies are to be attempted in human medicine. One of the current unknowns of microvascular angiogenesis is the role of $P2Y_2$ receptor activity, which this study aims to investigate.

$P2Y_2$ Receptors

P2 receptors belong to the purinergic receptor family, which describes a group of membrane receptors that respond to nucleotides (Burnstock, 2018). P2Y and P2X receptors are different subtypes of purinergic receptors: P2Y receptors are G-protein coupled receptors, and P2X receptors are ligand gated ion channels (Burnstock, 2018; Strassheim et. al, 2020). Purinergic receptors are now known to respond to both purines and pyrimidines and have a variety of functions in the body, including inflammation, pain, vasodilation, tissue proliferation, and regeneration (Burnstock, 2018). Of the eight known P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$), there are different levels of each receptor in various types of vasculature and various locations of the body. Though there is extensive crosstalk—one cell communicating and signaling another cell—of cytokines and growth factors during angiogenesis, the $P2Y_2R$ activity is the most prominent in endothelial proliferation compared to the other P2Y receptors (Strassheim et. al, 2020).

$P2Y_2R$ specifically responds to adenosine triphosphate (ATP), uridine triphosphate (UTP), and dinucleotide uridine adenosine tetraphosphate (Up4A) (Zhou et. al, 2016). Up4A stimulation of vascular endothelial cells upregulated the mRNA of the $P2Y_2R$ as well as the angiogenic and lumen forming properties of the tissue in culture (Zhou et. al, 2016). In physiologic conditions, shear stress and hypoxia often lead to the release of ATP or UTP from endothelial cells, which may act to stimulate $P2Y_2$ receptors and lead to decreased vasotone (Erlinge & Burnstock, 2008). The $P2Y_2R$ has regulatory function in hypertension, vascular aging and remodeling, vascular inflammation, atherosclerosis, diabetic vascular disease, leukocyte infiltration and vascular permeability (Strassheim et. al, 2020; Erlinge & Burnstock, 2008). Many of these physiologic processes and pathologies need further research to be understood well. As quite recently identified to potentially have function in microvasculature, the activity of $P2Y_2R$ is not yet well characterized in microvascular angiogenesis and proliferation.

Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2)

Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) is a common

marker of angiogenesis and proliferation in microvascular cells, so this study aims to assess the effect of $P2Y_2R$ on the expression of VEGFR-2 to better understand $P2Y_2R$'s role in microvascular angiogenesis and proliferation. VEGFR-2 is a receptor tyrosine kinase which responds to various signals, notably VEGF ligands. During angiogenesis, the specialized tip of the endothelial cells use the abundantly expressed VEGFR-2 on their filopodia to detect areas lacking vascularization. When VEGF ligands bind to VEGFR-2, autophosphorylation occurs and the increased kinase activity results in an increase in mitotic and survival signaling. Additionally, this signal transduction event can lead to the activation of several proteins that impact angiogenesis, including phospholipases, other kinases, motor proteins, and cell adhesion molecules (Abinhand et. al, 2016). The tip cells are generally characterized by their high expression of VEGFR-2 as well as their lack of vascular lumen and proliferation (Motherwell et. al, 2018) The other endothelial phenotype, the stalk cells, are the ones that are forming the vascular lumen, proliferating, and structurally supporting the tip cells (Abhinand et. al, 2016; Motherwell et. al, 2018). Without the proper function of these phenotypes, angiogenesis cannot be properly carried out both physiologically and in tissue engineering biomimetic models, emphasizing their importance in microvascular function (Abhinand et. al, 2016; Motherwell et. al, 2018; Wu et. al, 2017).

In cultured endothelial cells isolated from arterial tissue, activation of $P2Y_2R$ increased Rac1 activity, forming a complex of angiogenic regulators VE-cadherin and VEGFR-2 with the $P2Y_2R$. This study suggests $P2Y_2R$ and VEGFR-2 interact during UTP stimulation in endothelial cells (Liao et. al, 2014). In another study, $P2Y_2R$ KO mice had significantly decreased arteriogenesis following arterial injury and ischemia. Unexpectedly, this study also found that the $P2Y_2R$ KO mice had significantly increased angiogenesis, opposing the reduced arteriogenesis finding (McEnaney et. al, 2016). These findings point to $P2Y_2R$ as a fine regulator of the macrovascular and microvascular environmental stress response, especially in arteriogenesis and angiogenesis.

Additionally, stimulated $P2Y_2R$ upregulates VCAM expression via VEGFR-2 transactivation (Erlinge & Burnstock, 2008). Interestingly, upregulated $P2Y_2R$ has shown to lead to constitutive phosphorylation of VEGFR-2, but an overall decrease in expression level of VEGFR-2 in microvasculature. Moreover, upregulated $P2Y_2R$ expression leads to an increase of proangiogenic endothelial cell phenotype but reduction in responsiveness to the VEGF ligand (Müehleder et. al, 2020). This process is likely regulated by an extensive array of related receptors, including VEGFR-1, VEGFR-3, other of the various P2YRs, other endothelial adhesion molecules such as VE-Cadherin,

and circulating nucleotides (Müehleder et. al, 2020). This study aims to quantify the expression of VEGFR-2 modulated by $P2Y_2R$ using microvascular endothelial cells (MEC) derived from WT and $P2Y_2R$ KO mice.

Microvascular Endothelial Cells

Microvascular endothelial cells (MEC) "are multifunctional cells that line the vessels of the circulatory system," according to Lifeline® Cell Technology. Endothelial cells are lining the innermost layer of the vascular wall, protecting and creating a barrier for the vascular system as well as interacting with factors in the bloodstream. Interaction with these factors by the microvascular endothelial cells help mediate wound healing and inflammatory response of the body (Technology, Lifeline® Cell, and Lifeline Cell Tech Team). For this research, we use MEC, which represents small vessels in the vasculature including capillaries. MEC can be used to study functions such as the formation of a basement membrane, angiogenesis, and attachment/migration of lymphocytes through endothelial monolayers (Karasek, 1989). Using normal MEC and MEC deficient $P2Y_2R$ as cell models, this study will test the hypothesis that UTP stimulation would increase the level of VEGFR-2 expression in WT-MEC, but not in $P2Y_2R$ KO-MEC.

Materials and Methods

Isolation and Culture of Microvascular Endothelial Cells

The isolation and culture of MEC has been previously reported (Wang, et. al, 2020). In brief, abdominal skeletal muscle was harvested from four WT or $P2Y_2$ KO mice and cut into 0.5mm fragments. The animal care and use were conducted in accordance with the Office of Research Administration at Missouri State University. The endothelial cells were isolated using digestion solutions and Dynabeads™ (spherical beads with magnetic properties) coated with GS-I (L-2380, Sigma Aldrich, St. Louis, MO). The isolated cells were further extracted using Dynabeads™ coated with monoclonal antibodies against CD-31/PECAM-1 (BD Biosciences, San Jose, CA). The cells were cultured and validated with endothelial cell function and markers.

Tissue Culture

Isolated MEC were seeded into t-25, t-57, or 6-well culture plates coated with 0.02% gelatin. The culture medium was made with DMEM-F12 (Gibco, Waltman, MA) containing 10% fetal bovine serum (FBS; vol/vol %), endothelial cell growth supplement (50 μg/mL Corning Life Science, Bedford,

MA), heparin (5 U/mL, Sigma Aldrich, St. Louis, MO), antimycotic-antibiotic solution (10 µl/mL, Gibco, Waltman, MA), and L-glutamine (0.1 mg/mL, Gibco, Waltman, MA).The cells were maintained at 37° Celsius, 5% CO_2, and 90% humidity.

Optimization of VEGFR-2 Antibody

MEC were seeded into two t-75 flasks, grown to 85% confluency, and washed with DPBS (a balanced salt solution). The cells were removed from the gelatin using 0.05% Trypsin in EDTA (Gibco, Waltman, MA) and washed with MEC culture medium. The cells were aliquoted into Eppendorf tubes and incubated in 250 µL of 4% paraformaldehyde at 4° C for 20 minutes. The cells were washed in a staining buffer composed of 5% Bovine Serum Albumin ((BSA) Acros Organics, Lot# B0144435) in diphosphate buffered solution ((DPBS) Thermofisher, Waltman, MA). The pellets were dislodged with 100µL DPBS. Ice-cold methanol (900 µL) was added to the cells, and they were incubated for 30 minutes at 0° C to permeabilize the cells. The cells were washed with staining buffer and incubated in Mouse VEGFR2/KDR/Fik-1 PE-conjugated Antibody (R&D Systems, clone #522302, catalog #FAB4432P) concentrations overnight at 4° C, protected from light. The cells were washed in staining buffer and were run through flow cytometry analysis.

UTP Stimulation Flow Cytometry Analysis

2 mL of 10-5 M UTP in DPBS were added to the cells grown up to 90% confluency and incubated at 37° C, 5% CO_2, and 90% humidity for 15 minutes, 3 hours, or 6 hours. The cells were removed from the wells using 2 mL of Enzyme Free Cell Dissociation Solution (Sigma Aldrich, St. Louis, MO), and then fixed with 250 µL of 4% paraformaldehyde for 20 minutes at 4° C. The cells were washed with 5% BSA in DPBS staining buffer, and the pellet was dislodged with 100 µL of DPBS. Ice-cold methanol (900 µL) was added to the cells followed by incubation at 0° C for 30 minutes to permeabilize the cell. The cells were incubated with 5 µg/µL of VEGFR2/KDR/Fik-1 PE-conjugated Antibody (R&D Systems, clone #522302, catalog #FAB4432P) overnight at 4° C, protected from light. The cells were washed with staining buffer and were run through flow cytometry analysis.

Flow Cytometry Data Analyais

The flow cytometry data was analyzed using the Accuri C6 Flow Cytometer (Bd Biosciences, San Jose, CA). The initial gating of the cell population used FSC-A/SSC-A, where all debris events were excluded in the analysis

population in a fluorochrome independent gating. The Mouse VEGFR2/KDR/Fik-1 antibody is conjugated with PE Fluorochrome and was analyzed with 488nm laser lines and 525/72 emission filters. Therefore, the median FL2-H was compared between samples as it corresponds to PE Fluorochromes and is in direct relationship to the intensity of PE present on the cell. The median FL2-H values were used. Accuri C6 Flow Cytometer also provided the cell count, percent of population, and events measured during flow cytometry.

Matrigel Assay

Tubes of Matrigel (BD Biosciences Company) were thawed overnight at 4° C. Each well of pre-cooled 96 well plate was coated with 50 µl of Matrigel followed by incubation at 37° C for one hour. The WT MECs in 100 µl were seeded per well. The capillary-like tube formation was assessed in the presence and absence of 20 µl of UTP (10-5M) per well. Imaging of tube formation was acquired using an inverted microscope (Olympus CKX41) with 10x magnification lens as the cells were grown on the Matrigel for 2, 4, 6, 9, 12, and 24 hours.

Analysis of Capillary-like Tube Formation and Cell Proliferation

The images of the cell proliferation and capillary-like tube formation at various time intervals were analyzed using Image J software, which is publicly available from the National Institutes of Health website. The area of cell proliferation was measured by circling the cluster of cells surrounding the capillary-like tube structure (*Figure 1A*). The length of the tubes was measured by drawing a line running down the length of capillary-like tube formation. Initially, all cell area and tube length were measured in digital pixels. These values were further converted into corresponding units: μm^2 for area and µm for length, based on a scale obtained using the microscope calibration slide. Each experimental value (n = 1) was obtained from the average of triplicate samples (three wells). The area of the cell and length of the capillary-like tubes that formed were analyzed. The scale was determined by using the 10x lens of the inverted microscope by the 96 well plate cover.

Statistical Analysis

In the graphical and statistical analysis, all values are expressed as mean ± SEM. The one-way ANOVA and unpaired t-tests were used to determine the p values using GraphPad Prism (Graph Pad Software, San Diego, CA). P value less than 0.05 is considered statistically significant in this study. The VEGFR-2 Expression Index (EI) was calculated by multiplying the median

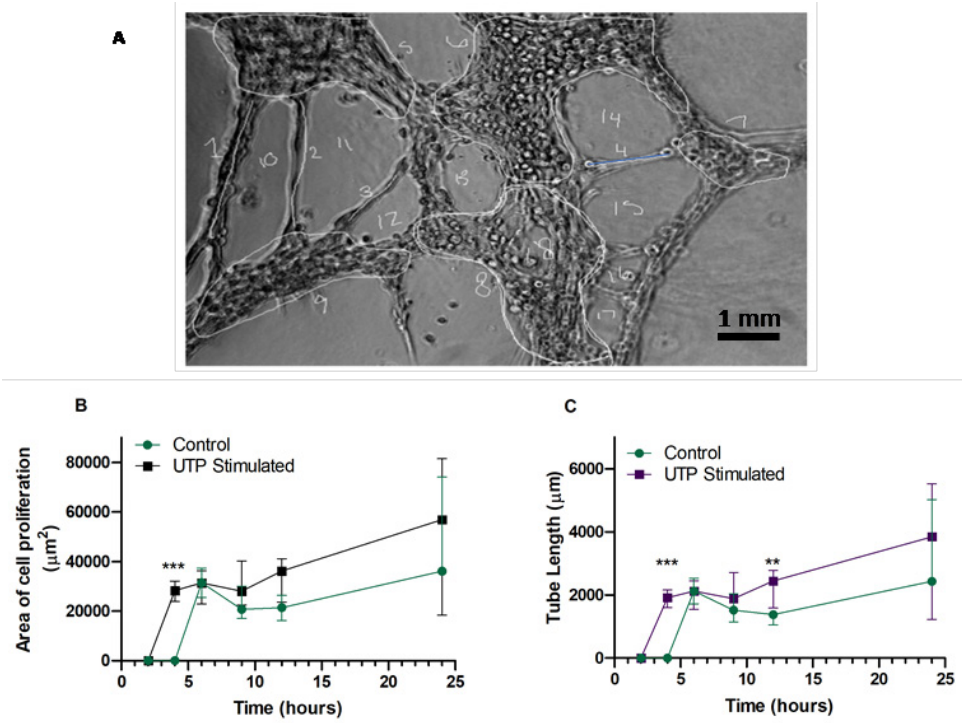


Figure 1: Cell proliferation and capillary-like tube formation in the presence of UTP in WT MEC. A representative image of cell proliferation and capillary-like tube formation in WT MEC cells stimulated by UTP for nine hours (*A*). The white circles were the clusters of cells that were measured for the area of cell proliferation, and the white lines on the image indicate the lengths of the capillary-like tubes that formed and were measured. All images were analyzed using Image J software. The area of cell proliferation (*B*) and length of tubes (*C*) increased the time period of UTP stimulation. Stimulated cells with UTP (purple square) exhibited greater area of cell proliferation at four hours and tube length at four and 12 hours compared with unstimulated cells (green dot). Each value was obtained from the average of triplicate wells (n = 1) and the experimental replication was 3 ($n = 3$).
$^{**}p < 0.01$ and $^{***}\ p < 0.001$.

Optimization of [VEGFR-2 Antibody] in WT Cells

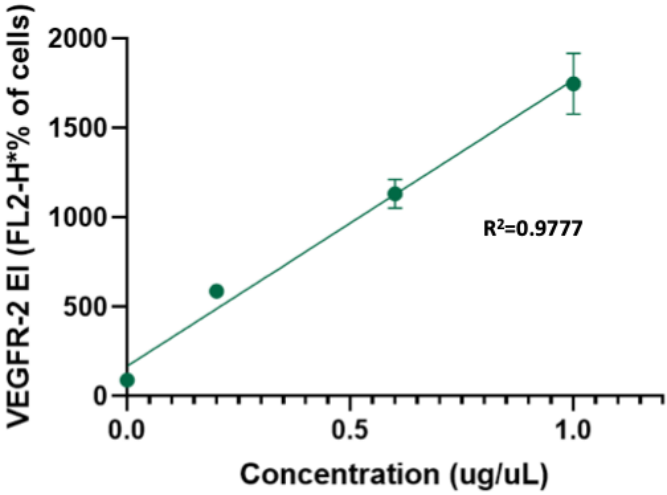


Figure 2: The optimization of Mouse-VEGFR2/KDR/Fik-1 PE-conjugated antibody for further use in flow cytometry analysis. Concentrations of 0.2, 0.6, and 1.0 µg/µL were tested and 1.0 µg/µL concentration was selected for further experiments. The R^2 value of the linear trendline is 0.9777. The correlation coefficient (r) = 0.989.

FL2-H by the percent of cells positively stained compared to the total cell population.

Results

Matrigel Assay: Capillary-Like Tube Formation and Cell Proliferation

Increased area of cell proliferation and capillary-like tube formation over time between two and 24 hours in WT MEC stimulated with UTP tended to be faster than those in unstimulated cells (*Figure 1*). Cell proliferation (*Figure 1B*) and tube formation (*Figure 1C*) began at two hours for stimulated cells and four hours for unstimulated cells after they were seeded in Matrigel. The area of cell proliferation at four hours and length of formed tube at four and 12 hours were greater in UTP-treated MEC than untreated MEC ($n = 3$, $p < 0.01$).

Optimization of VEGFR-2 Antibody Flow Cytometry Assay

VEGFR-2 primary antibody is directly labeled with PE. To determine the optimal concentration of the antibody, we mea-

FL2-H vs Cell Count of WT and P2Y2 KO Cells

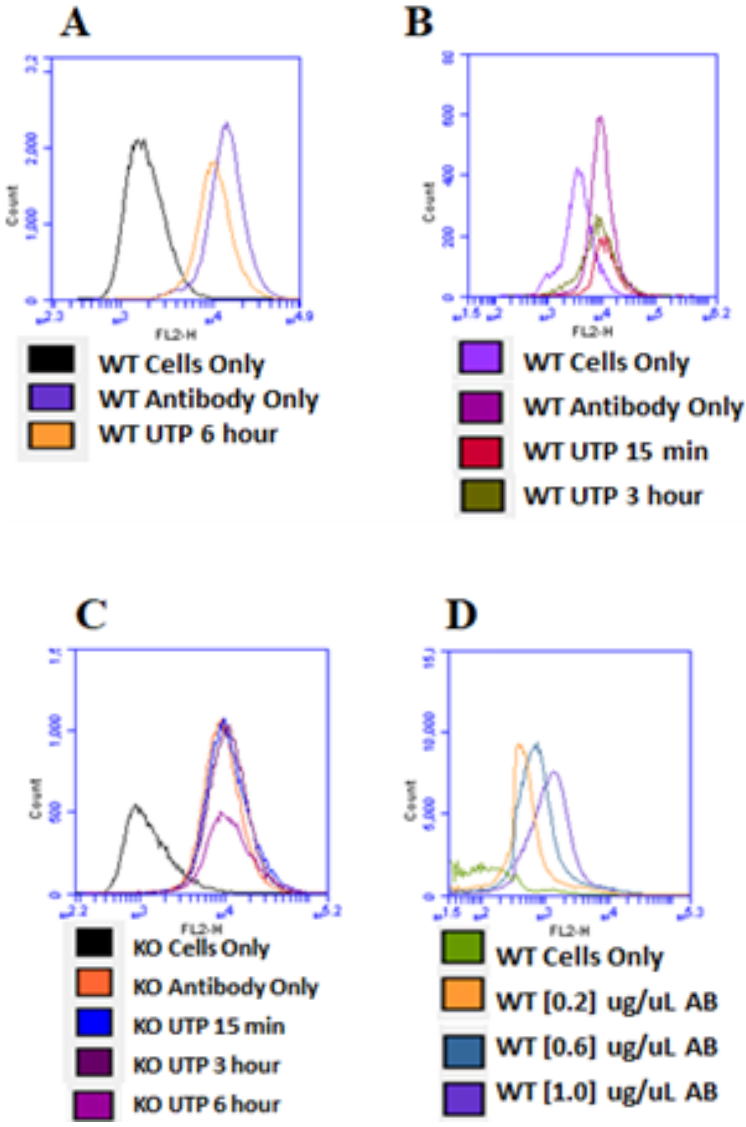


Figure 3: The representative cell count vs. FL2-H in primary cultured MEC derived from WT and P2Y2 KO mice under various conditions. Cell count in the cell population under control (cells only), incubated with antibody, and treated with UTP and antibody revealed VEGFR-2 expression in WT cells *(A and B)* and KO cells *(C)*. We performed experiments using WT MECs in the presence of different concentrations of antibody against VEGFR-2 (0, 0.2, 0.6, and 1.0 μg/μL) to determine optimal concentration of antibody for the study *(D)*.

sured percent of cell population bound with the antibody and fluorescence intensity in each WT cell sample. Four samples of WT MEC were incubated with VEGF-R2/KDR/Flk1 antibody at the concentration of 0 (control), 0.2, 0.6, and 1.0 μg/μL, respectively. The expression index (EI) was calculated

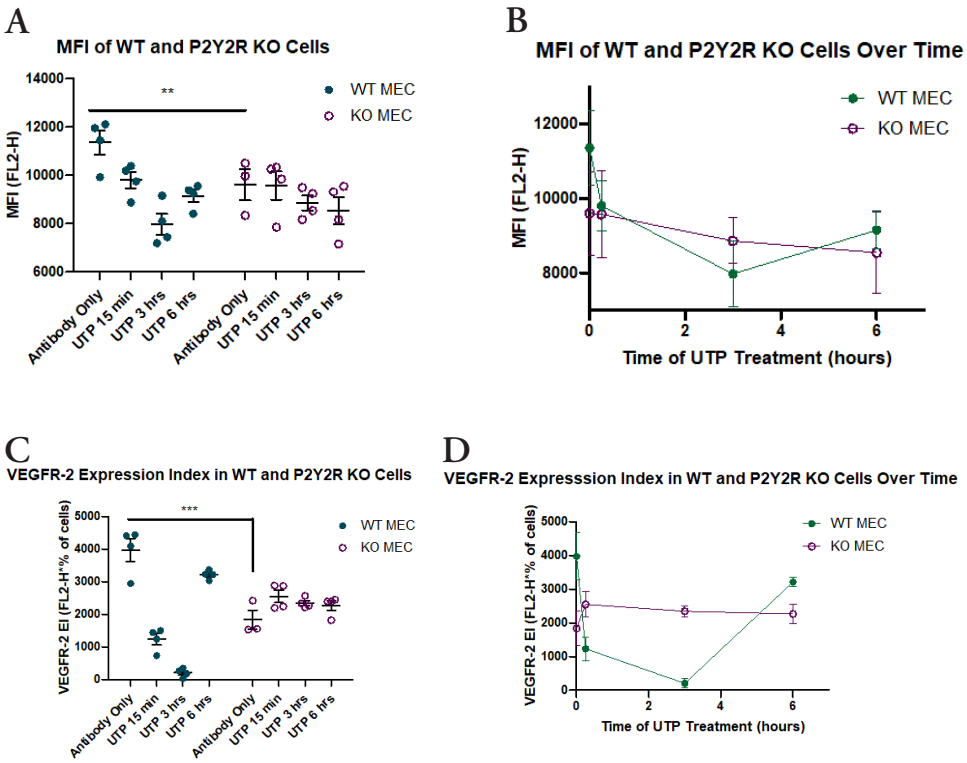


Figure 4: VEGFR-2 fluorescent intensity and expression index in WT and $P2Y_2R$ KO MEC. VEGFR-2 expression was measured by flow cytometry assay. The median fluorescence intensity (MFI) of VEGFR-2 expression in WT and $P2Y_2$ KO cells was normalized by subtracting background MFI derived from the cells without antibody incubation. Expression index (EI) is a product of MFI and percent of cells positively stained in a MEC population. The baseline VEGFR-2 expression decreased in P2Y2 KO MEC in the comparison with the baseline of WT MEC. MFI (*A and B*) and EI (*C and D*) of VEGFR-2 expression decreased with UTP (10^{-5} M) stimulation at 15 minutes to 3 hours from the baseline in WT MEC ($p < 0.01$) and did not change before and after UTP treatment in $P2Y_2$ KO MEC. At 6 hours, EI elevated compared with three hours in WT MEC stimulated with UTP ($p < 0.01$). Each value was expressed as mean ± SEM from 3–4 separated experiments ($n = 3–4$). One-way ANOVA and student t-test were used. P value < 0.05 was considered statistical significance. The symbol ** indicates $p < 0.01$.

using FL2-H multiplied by the percent of cells bound with antibody in the population. The value of EI was strongly correlated with concentration of the antibody (r^2 = 0.98, *Figure 2*). Based on our data and the manufacturer's recommendation, we decided to use the concentration of 1.0 μg/μL for the study.

VEGFR-2 Expression of MEC

We assessed VEGFR-2 expression in MEC treated with UTP (10^{-5} M) in both WT and $P2Y_2R$ KO cells using flow cytometry assay. The representative plots of the cell count versus fluorescence intensity in WT and KO MEC are shown in *Figure 3*. The fluorescence intensity (FI) was expressed in the median of the value (MFI). The MFI of the cells without VEGF-R2/KDR/Flk1 antibody incubation was defined as the background MFI for the cells. MFI of

	Median FL2-H	% of all cells	VEGFR-2 expression index
Cell only	1765	35.6%	628.2
	1816	37.1%	673.4
	1774	36.6%	649.1
	3652	18.6%	679.4
Antibody only	13223	35.7%	4719.3
	13931	36.5%	5086.2
	11687	30.2%	3529.5
	13743	37.2%	5117.9
UTP 15 minutes	12166	16.7%	2029.2
	11565	18.9%	2183.5
	11954	15.6%	1867.2
	10662	13.8%	1405.3
UTP 3 hours	9211	9.3%	852.0
	9916	10.4%	1031.3
	10923	7.4%	806.1
	8976	7.8%	701.0
UTP 6 hours	11154	35.5%	3955.2
	11373	32.6%	3711.0
	11013	35.0%	3857.9
	10193	38.1%	3886.6

Table 1: The Median FL2-H, % of all cells, and VEGFR-2 Expression Index in WT condtions: Cells Only, Antibody Only, UTP 15 minutes, UTP 3 hours, and UTP 6 hours. VEGFR-2 Expression index was calaculated by multiplying the Median FL2-H by the percent of all cells. The Median FL2-H represents the Median Fluoresent Intensity, which is shown in *Figure 2*. The VEGFR-2 Expression Index is shown in *Figure 3*.

VEGFR-2 expression in each sample incubated with the antibody was normalized by subtracting background MFI.

The basal VEGFR-2 expression in WT MEC was higher compared with basal expression in KO MEC ($p < 0.01$). VEGFR-2 expression in WT MEC treated with UTP (10^{-5} M) decreased with increasing incubation period from 15 minutes to three hours ($n = 3–4$, $p = 0.002$). There was a trend increase in MFI at six hours compared with MFI at the three-hour incubation period, but the difference was not significant ($n = 3–4$, $p = 0.08$). In contrast, the expression of VEGFR-2 in KO MEC was not changed before and after UTP treatment (*Figure 4A*). The dynamic changes in VEGFR-2 with the incubation period in both types of MEC are shown in *Figure 4B*.

The VEGFR-2 expression index (EI) is determined by multiplying the MFI by the percent of cells included in the analysis population compared to the entire cell population (*Table 1, Table 2, Figure 3*). Analysis of the ef-

	Median FL2-H	% of all cells	VEGFR-2 expression index
Cells only	1168	11.1%	130.2
	1186	11.3%	133.5
	1300	12.6%	163.9
Antibody only	11133	14.9%	1661.0
	11688	21.9%	2553.8
	9641	17.8%	1719.0
UTP 15 minutes	11427	26.3%	3004.1
	9039	25.8%	2327.5
	11636	20.7%	2405.1
	11636	27.5%	3043.7
UTP 3 hours	9702	24.7%	2292.5
	10678	23.1%	2467.7
	9464	25.0%	2370.7
	10461	26.1%	2729.4
UTP 6 hours	10710	24.1%	2579.0
	10502	24.1%	3532.0
	8453	23.5%	1982.2
	9382	27.2%	2551.9

Table 2: The Median FL2-H, % of all cells, and VEGFR-2 Expression Index in $P2Y_2$ KO conditions: Cells Only, Antibody Only, UTP 15 minutes, UTP 3 hours, and UTP 6 hours. VEGFR-2 Expression index was calaculated by multiplying the Median FL2-H by the percent of all cells. The Median FL2-H represents the Median Fluoresent Intensity, which is shown in *Figure 2*. The VEGFR-2 Expression Index is shown in *Figure 3*.

fect of UTP stimulation on VEGFR-2 receptor expression in WT and KO MEC was quantified using EI. Like the MFI analysis, higher basal levels of VEGFR-2 in KO MEC relative to WT MEC (*Figure 4*) and the decrease in EI with increasing UTP treatment period from 15 minutes to 3 hours were observed. However, EI analysis further revealed significant elevation of VEGFR-2 expression at 6 hours relative to 3 hours of UTP treatment ($p < 0.01$) as shown in *Figure 4*.

Discussion

This study aimed to determine the effect of UTP stimulation on the formation of capillary-like tube structures in MEC. We found UTP promoted MEC proliferation and formation of capillary-like tubes. We further determined VEGFR-2, a key angiogenic signaling molecule, mediates UTP-activated $P2Y_2R$. In the study, we used flow cytometry assay to measure expression of VEGFR-2 using $P2Y_2R$ KO MEC model. The findings demonstrated the $P2Y_2R$ KO mice did not show a significant change in VEGFR-2 in response to UTP while the WT-MEC did exhibit a response to UTP stimulation. The results are partially consistent with our hypothesis.

In the Matrigel assay, we showed UTP stimulation on $P2Y_2R$ causes capillary tube-like formation in MEC more quickly and efficiently. Previous studies have demonstrated similar results, where nucleotide activation of P2 receptors leads to increased capillary tube-like formation (Rumjahn et. al, 2009) There were time periods where the WT MEC was above or near the same value as UTP stimulated WT MEC (*Figure 1*). This could be due to the small well of the 96 well plate in which the cells were contained, and that the UTP stimulated WT MEC were constricted and began to cluster. Another reason could be due to the desensitization of the $P2Y_2R$. Once the receptor was desensitized, the peak of activity leveled off or declined.

Following UTP stimulation for 15 minutes, WT-MEC decreased VEGFR-2 expression. Further decrease in VEGFR-2 expression was observed at three hours of UTP stimulation. These findings may be explained by the internalization of VEGFR-2 and the complex intracellular VEGFR-2 trafficking and signaling that follows internalization (Simons, 2012; Lampugnani et. al, 2006). It wasn't until 6 hours of UTP stimulation that the VEGFR-2 expression levels began to rise, but it remained lower than the basal expression. The findings of initial decrease in VEGFR-2 expression following $P2Y_2R$ activation are consistent with the recent findings of a study where the overexpression of $P2Y_2R$ lead to a decrease in VEGFR-2 expression (Müehleder et. al, 2020). Moreover, studies have shown that phosphorylation of VEG-

FR-2 increases although the level of VEGFR-2 expression decreases during nucleotide stimulation (Müehleder et. al, 2020; Rumjahn et. al, 2009). The evidence implicates the activity of VEGFR-2 in angiogenesis may be independent of the level of VEGFR-2 expression. The major biologic function of VEGFR-2 signaling is angiogenesis. Additionally, internalized VEGFR-2 can become phosphorylated, which connects the two possible explanations of this result: receptor internalization and activity state (Lampugnani et. al, 2006).

$P2Y_2Rs$ are thought to undergo desensitization after stimulation, so this could potentially explain the late rise of VEGFR-2. However, receptor desensitization of $P2Y_2R$ is generally thought to be an immediate response and may not explain why VEGFR-2 rises after six full hours of $P2Y_2R$ stimulation. Moreover, desensitization of $P2Y_2R$ is not fully understood, especially in the context of the effect of $P2Y_2R$ on angiogenesis (Flores et. al, 2005). It is unclear without further investigation of the relationship of $P2Y_2R$ stimulation and VEGFR-2 expression why these results occurred. However, it is clear that the WT-MEC had a distinct VEGFR-2 expression response to the UTP stimulation and the KO-MEC did not. This strongly suggests $P2Y_2R$ does play a role in the mediation of VEGFR-2 expression and therefore could be an important regulator of microvascular angiogenesis.

Future studies need to be conducted to further understand the role of $P2Y_2R$ in microvascular angiogenesis. First, a study may be conducted to understand the effects of additional prolonged UTP stimulation on VEGFR-2 expression MEC, perhaps up to 12 or 24 hours of stimulation. This would identify the expression VEGFR-2 to further the understanding of UTP stimulation over time. Based on the results of this study, the result of prolonged UTP exposure may follow the trend for an increased expression of VEGFR-2 at 12 to 24 hours. If VEGFR-2 expression did reach the baseline value, it would further the understanding of how UTP alters VEGFR-2 expression over prolonged exposure. In this case, future studies should aim to assess if the expression remains constant or if it exceeds the baseline value. Additionally, future studies could examine the concentration of UTP stimulation on the response of VEGFR-2. This study used 10^{-5} M UTP because it is a pre-optimized EC50 for UTP in MEC. However, it would be helpful to see the effects of different doses of UTP on $P2Y_2R$ stimulation in angiogenesis. Finally, assessment of VEGFR-2 activity could better understand VEGFR-2 signaling in response to $P2Y_2R$ activation. VEGFR-2 activity can be determined through a measure of the phosphorylation state of VEGFR-2 using blotting assays. Though flow cytometry provides an interesting measure of the protein expression of VEGFR-2 in this study, more assays would provide a fuller view of $P2Y_2R$ in angiogenesis.

Future studies for the Matrigel assay could be using KO microvascular endothelial cells to identify the role of $P2Y_2R$ in angiogenesis. The data obtained from this study is preliminary data that needs to be optimized with further experimentation to determine the accuracy of the results. A way to improve this data would be to count the number of cells each time before seeding them in the Matrigel to determine if this is a constant control for the experiment. Another experiment would be using factors for $P2Y_2R$ stimulation and angiogenesis functions aside from UTP. A few factors to consider for MEC with $P2Y_2R$ would include VEGF and LPS. Treatment with LPS to the WT MEC has also shown upregulation of VEGF and promotion of angiogenesis and can be visualized with a Matrigel assay as well to compare images and measurements of capillary-like tube formation (Shin et. al, 2014). Another experiment that could be performed would be to use VEGF and UTP combined to stimulate VEGFR-2 in a Matrigel assay to increase angiogenesis, similar to a previous study that assessed P2Y receptors in angiogenesis (Rumjahn, 2009). Further determination in activation of angiogenesis can allow us to learn regulatory factors of this process in MEC and can allow us to use $P2Y_2R$ for stimulation or suppression of this pathway to treat different diseases and conditions in the body such as cancer, ischemic heart disease, and rheumatoid arthritis.

References

Abhinand, C. S., Raju, R., Soumya, S. J., Arya, P. S., & Sudhakaran, P. R. (2016). VEGF-A/VEGFR2 signaling network in endothelial cells relevant to angiogenesis. *Journal of Cell Communication and Signaling, 10*(4), 347–354. PMCID: PMC5143324

Burnstock, G. (2018). Purine and purinergic receptors. *Brain and Neuroscience Advances, 2.* https://doi.org/10.1177/2398212818817494

Erlinge, D., & Burnstock, G. (2008). P2 receptors in cardiovascular regulation and disease. *Purinergic Signalling, 4,* 1–20. https://doi.org/10.1007/s11302-007-9078-7

Flores, R. V., Hernández-Pérez, M. G., Aquino, E., Garrad, R. C., Weisman, G. A., & Gonzalez, F. A. (2005). Agonist-induced phosphorylation and desensitization of the P2Y2 nucleotide receptor. *Molecular and Cellular Biochemistry, 280,* 35–45. https://doi.org/10.1007/s11010-005-8050-5

Galiano, R.D., Tepper O. M., Pelo C. R., Bhatt, K. A., Callaghan, M., Bastidas, N., Bunting, S., Steinmetz, H. G., & Gurtner, G.C. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells (2004). *The American Journal of Pathology, 164*(6), 1935–1947. https://doi.org/10.1016/S0002-9440(10)63754-6

Karasek, M. A. Microvascular endothelial cell culture (1989). *Journal of Investigative Dermatology, 93*(2, Suppl.), 33S–38S. https://doi.org/10.1038/jid.1989.6

Lampugnani, M. G., Orsenigo, F., Gagliani, M.C., Tacchetti C., & Dejana, E. Vascular endothelial cadherin controls VEGFR-2internalization and signaling from intracellular compartments (2006). *Journal of Cell Biology, 174*(4), 593–604. https://doi.org/10.1083/jcb.200602080

Liao, Z., Cao, C., Wang, J., Huxley, V. H., Baker, O., Weisman, G. A., & Erb, L. The P2Y2 receptor interacts with VE-Cadherin and VEGF receptor-2 to regulate Rac1 activity in Endothelial cells (2014). *Journal of Biomedical Science and Engineering, 7*(14), 1105–1121. https://doi.org/10.4236/jbise.2014.714109

McEnaney, R. M., Shukla, A., Madigan, M. C., Sachdev, U., & Tzeng, E. P2Y2 nucleotide receptor mediates arteriogenesis in a murine model of hind limb ischemia (2016). *Journal of Vascular Surgery, 63*(1), 216–25. https://doi.org/10.1016/j.jvs.2014.06.112

Motherwell, J. M., Anderson, C. R. & Murfee, W. L. Endothelial cell phenotypes are maintained during angiogenesis in cultured microvascular networks (2018). *Scientific Reports, 8,* 5887. https://doi.org/10.1038/s41598-018-24081-z

Mühleder, S., Fuchs, C., Basíllo, J., Szwarc, D., Pill, K., Labuda, K., … & Holnthoner, W. Purinergic P2Y2 receptors modulate endothelial sprouting (2020). *Cellular and Molecular Life Sciences, 77,* 885–901. https://doi.org/10.1007/s00018-019-03213-2

Rumjahn, S. M., Yokdang, N., Baldwin, K. A., Thai, J., & Buxton, I. L.O. Purinergic regulation of vascular endothelial growth factor signaling in angiogenesis (2009). *British Journal of Cancer, 100*(9), 1465–1470. https://doi.org/10.1038/sj.bjc.6604998

Secomb, T. W., & Pries, A. R. Microvascular plasticity: Angiogenesis in health and disease – Preface (2016). *Microcirculation, 23*(2), 93–94. *OpenAIRE.* https://search.ebscohost.com/login.aspx?direct=true&AuthType=sso&db=edsair&AN=edsair.od.....267..321a5fefae5f246bc-35790c45508b497&site=eds-live&scope=site&custid=039-820

Shin, M. R., Kang, S. K., Kim, Y. S., Lee, S. Y., Hong, S. C., & Kim, E. C. TNF-α and LPS activate angiogenesis via VEGF and SIRT1 signalling in human dental pulp cells (2014). *International Endodontic Journal, 48*(7), 705–16. https://doi.org/10.1111/iej.12396

Simons, M. An inside view: VEGF receptor trafficking and signaling. *Physiology (Bethesda) 27*(4), 213–222. https://doi.org/10.1152/physiol.00016.2012

Strassheim, D., Verin, A., Batori, R., Nijmeh, H., Burns, N., Kovacs-Kasa, A, … & Gerasimovskaya, E. P2Y purinergic receptors, endothelial dysfunction, and cardiovascular diseases (2020). *International Journal of Molecular Sciences, 21*(18), 6855. https://doi.org/10.3390/ijms21186855

Technology, Lifeline® Cell, and Lifeline Cell Tech Team. "Microvascular endothelial cells circulatory research." *Lifeline Cell Technology*, www.lifelinecelltech.com/microvascular-endothelial-cells-circulatory-system-research/#:~:text=

Wang, J., Harvey, J., Garrad, R., Huxley, V., Erb, L., & Weisman, G. (2020). Simple, reliable isolation, purification and cultivation of murine skeletal muscle microvascular endothelial cells. *Journal of Biomedical Science and Engineering, 13*(12). 290–299. https://doi.org/10.4236/jbise.2020.1312026

Wu, W., Duan, Y., Ma, G., Zhou, G., Park-Windhol, C., D'Amore, P. A., & Lei, H. AAV-CRISPR/Cas9-mediated depletion of VEGFR2 blocks angiogenesis in vitro (2017). I*nvestigative Ophthalmology & Visual Science, 58*(14). 6082–6090. https://doi.org/10.1167/iovs.17-21902

Zhou, Z., Chrifi, I., Xu, Y., Pernow, J., Duncker, D. J., … & Cheng, C. Uridine adenosine tetraphosphate acts as a proangiogenic factor in vitro through purinergic P2Y receptors (2016). *American Journal of Physiology, 311*(1). H299–309. https://doi.org/10.1152/ajpheart.00578.2015

About the Authors

Molly Del Rossi is a junior Creative Writing major and a Journalism minor. She is currently pursuing distinction in her major at Missouri State University. Her literary interests include horror and stream of consciousness. She hopes to be an artist-in-residence in her future, traveling across the world and teaching what she loves.

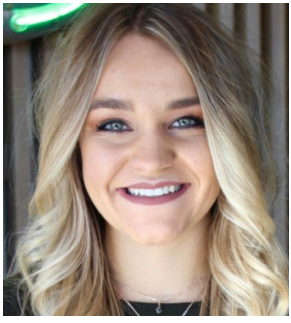
Abigail Jensen is a junior at Missouri State University. She is double majoring in Creative Writing and Theatre. She is originally from St. Louis. She gives tours on campus as a University Ambassador and is an Assistant Poetry Editor for Moon City Review. At Missouri State, she has been seen on stage in both main stage and student-led productions.

Kyle Hulsebus is a senior Vocal Music Education major. Although his talents lie in the field of education, he enjoys researching and performing music by underappreciated composers. Upon graduation, he intends to start teaching in South-Central Missouri while pursuing a Master of Music Education degree at the University of Missouri.

Whitney Mosley is a graduate of Missouri State University, where she obtained her Bachelor of Arts Degree in Art History. She has presented her research in the MSU 14th Annual Art History Symposium and the NCSA 42nd Annual Conference: Discovery. Whitney plans to continue her studies and attend graduate school, aspiring to work as a museum educator.

Hannah Fuller graduated with a B.S. in History from Missouri State University in May 2021. She is currently working towards her MA in History at MSU, with research interests in public history, oral history, and LGBTQ+ studies. Following her Masters, she hopes to join a doctoral program and continue to pursue her historical interests through academic research and public outreach.

Casen Zelch is a senior History major. He plans to attend graduate school to pursue a Master of Arts degree in History, with an interest in LGBTQ+ history and culture. He hopes to work at a LGBTQ+ historical museum or archive and to continue research on queer history.



Emily Chisum is a current first year medical student at the University of Missouri School of Medicine. She graduated with a degree in Cell and Molecular Biology from Missouri State University in the spring of 2021. She is currently interested in pursuing a career in rural healthcare as a physician and takes a special interest in women health studies.

Richard Garrad, PhD, is a cell biologist who is particularly interested in the P2 nucleotide. His work has been funded by the NIH, and he has published over 25 papers. Dr. Garrad teaches Molecular Cell Biology, Virology and Senior Seminar and has advised many great students over the years, including Dr. Molly Greenwade and soon-to-be Dr. Emily Chisum.

Molly Greenwade, MD, is a gynecologic oncologist. She participates in clinical science research and works with medical students on their third-year clerkship rotations. Her research interests include gynecologic malignancy, targeted therapies, and the tumor microenvironment. She hopes to continue to build the clinical trial programs for gynecologic cancer patients of the Ozarks.

Tyler Swearingin graduated Summa Cum Laude in the MSU class of 2019 with a degree in Psychology along with Honors recognition. He is currently a first-year medical student at the University of Missouri. He will be graduating with his medical degree in the spring of 2024 where he then plans to attend residency in a surgical specialty.

Rachel Ingram is a senior Cell and Molecular Biology major. She plans to attend medical school in the fall of 2021 after graduating from Missouri State University. She enjoys her role as a tutor for human genetics and hopes to continue educating others by working at an academic medical center in the future.

Leanna Zelle graduated from Missouri State University with honors distinction in Cell and Molecular Biology. She is now a first year medical student at the University of Missouri - Columbia. She plans to continue researching in medical school and hopes to pursue a surgical subspecialty in the future.

Abby Gann graduated from Missouri State University with honors distinction in Cell and Molecular Biology. She is currently working at Cox Health as a Patient Throughput Facilitator and is an assistant coach for the Hillcrest High School women's basketball team. She plans to apply to medical school this fall in the hopes of pursuing her goal to become a physician.

Jianjie Wang, MD, PhD, earned an MD in Tianjin Medical University and a PhD in medical physiology at University of Missouri-Columbia. She has served in the department of Biomedical Sciences at Missouri State University as a faculty member for more than 10 years. Dr. Wang's research interests are focused on vascular biology, particularly microcirculation.

Want to submit to *LOGOS?* Visit missouristate.edu/logos for submission guidelines and instructions.

The generosity of the Honors College community enables our students to present original scholarship at national research conferences, to participate in study away programs, and to produce and publish *LOGOS: A Journal of Undergraduate Research*. Your contributions support our continuing efforts to provide the undergraduate students of Missouri with the outstanding academic and developmental opportunities that are hallmarks of an honors education. Visit www.missouristate.edu/honors to learn how your tax-deductible donation can directly support Honors College students.

Connect with us on social media and keep up-to-date on all *LOGOS* news!