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Ethics of Artificial Intelligence in Society

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ABSTRACT

Every day, artificial intelligence (AI) is becoming more prevalent as new technologies are presented to the public with the intent of integrating them into society. However, these systems are not perfect and are known to cause failures that impact a multitude of people. The purpose of this study is to explore how ethical guidelines are followed by AI when it is being designed and implemented in society. Three ethics theories, along with nine ethical principles of AI, and the Agent, Deed, Consequence (ADC) model were investigated to analyze failures involving AI. When a system fails to follow the models listed, a set of refined ethical principles are created. By analyzing the failures, an understanding of how similar incidents may be prevented was gained. Additionally, the importance of ethics being a part of AI programming was demonstrated, followed by recommendations for the future incorporation of ethics into AI. The term "failure" is specifically used throughout the paper because of the nature in which the events involving AI occur. The events are not necessarily "accidents" since the AI was intended to act in certain ways, but the events are also not "malfunctions" because the AI examples were not internally compromised. For these reasons, the much broader term "failure" is used.

KEYWORDS

Ethics; Artificial Intelligence; Agent-Deed-Consequence (ADC) Model; Principles of Artificial Intelligence; Virtue Ethics; Deontology; Consequentialism; AI Systems

INTRODUCTION

Artificial Intelligence (AI) is becoming more prevalent in society. As its outreach is becoming greater, the need for understanding how to avoid AI shortcomings is pertinent for developing quality applications in the future. Three contemporary examples of AI-related failures and failures are detailed. The relation of the examples to the principles of ethics in AI, the three ethical theories, and the Agent Deed Consequence Model (ADC Model) are analyzed. The discussion and analysis will lead to a greater understanding of ethics in AI, as well as provide useful societal applications. Throughout this research, there is a determination of how each failure occurred and how the failures could be prevented in the future. A system of several steps is proposed with the intention of ensuring AI systems remain ethical. It is hoped that this model will lead to a greater understanding of why AI commits mistakes in order to allow knowledge to lead towards better outcomes and the prevention of AI failures.

METHODS AND PROCEDURES

There is a growing demand for products with integrated AI. This demand brings out a need to ensure AI acts ethically. AI is a system that can operate and complete its designated tasks without direct human input. As AI does not have the same capabilities for morality and ethics as humans do, it is always possible that AI may unintentionally violate ethical boundaries. To determine whether AI acted ethically or not, three ethical theories used to determine the morality of humans were explored: virtue ethics, deontology, and consequentialism. In addition, Dubljević's nine proposed ethical principles for AI were used, 'fairness and non-discrimination', 'privacy, safety and security', 'human control of technology', 'transparency and explainability', 'accountability', 'promotion of human values', 'professional responsibility', and 'sustainable development'. The nine principles relate back to aspects of the three ethical theories as well. Using these guidelines, case studies were conducted on three AI failures detailing how they violated specific principles of ethics in AI. An analysis is conducted to show how similar incidents could be avoided in the future using the ADC model.

ETHICAL THEORIES

Three well-known ethical theories that are used to evaluate the ethics and morality of a situation were investigated. These three theories are virtue ethics, deontology, and consequentialism.

Ethical Theories				
Virtue Ethics:	Deontology:	Consequentialism:		
Emphasizes the character of a person. It does not attempt to identify singular moral principles to any situation, rather, each person and situation should be evaluated individually.	Emphasizes whether the actions of a person are right or wrong, and whether those actions respect obligations, duties, and rights in a given situation.	Focuses on whether the outcomes of a situation are morally correct or not. Related to utilitarian ethics which favors the option that will result in the most good.		

Table 1. Ethical Theories defined within a table.

Virtue Ethics

Virtue ethics is a broad model that emphasizes the agency or character of a person.^{3,4} The theories of virtue ethics, "do not aim primarily to identify universal principles that can be applied in any moral situation," unlike deontology and consequentialism theories.^{3,4} Virtue ethics would be concerned with examining virtues such as "responsibility", "righteousness", and "justice". These virtues would serve as motives for why an agent may have acted the way they did. Another way of thinking about virtue ethics would be to consider more than just the actions one took, but also the principles behind one's actions.⁵ A virtue ethics philosopher would believe that the reason for which a moral agent goes along with certain actions would be more important than the actions themselves.

Deontology

There are two components of deontology. The first is concerned with the actions of agents themselves and whether those actions are simply right or wrong.^{6,7} The second component is concerned with whether the actions of the agent respect the obligations, duties, and rights given the situation.⁸ Deontology claims that an agent is moral if it follows these two aspects. Ways to explore deontology ethics would be to identify the specific actions committed by an agent or certain expectations being followed through. Immanuel Kant was one such philosopher who supported this moral basis.⁵ Servicing patients in medicine and dentistry can serve as an example to further explain the point. In Washington, debates occurred on whether healthcare workers should be required to get the Covid-19 vaccine for work and if patients should also feel obligated to have the vaccine before going to a healthcare facility.⁵ Deontological ethics would examine whether it is right or wrong or set vaccines as mandatory in the healthcare system. To a deontologist, it would be wrong to restrict freedom for the sake of the greater good.⁵ In this sense, it would be wrong to mandate vaccinations to healthcare workers and patients.

Consequentialism

Consequentialism focuses on the results of an action, reasoning that an agent is moral if it chooses the most ethical consequence.⁸ Also known as utilitarian ethics, consequentialism seeks for a situation to yield the most positive outcomes.⁸ Using the same example as before, it is possible to view the same problem from a different ethical lens. Since the consequentialist viewpoint favors the "greater good", a philosopher would argue that it is right to set vaccines as mandatory in the healthcare system. As long as the vast majority of people who received the vaccine did not experience major negative side effects to it, then it would be reasonable to conclude that the greater good prevails over the individual, from the perspective of a consequentialist philosopher.

Philosophers and ethicists have used these three theories to determine the most ethically correct solution regarding specific ethical dilemmas. There cannot be a "most ethical course of action" in certain events because people would have their own opinions on what an AI should do given the context of the situation. A well-known example would be the Trolley Problem: philosophers such as Kant and Mill would have their own opinion on whether the AI driving the Trolley should steer one way or stay on course. Kant would argue that it is up to humans to decide whether the trolley should continue on its course or should be intercepted by a person while Mill would most likely argue that it is better for five people to be saved than one person as is in accordance with the utilitarian point of view. These theories will be utilized through the analysis of the ADC model in section five of the paper to decide what is right and wrong in different aspects of a situation.

PRINCIPLES OF ETHICAL AI

There are nine principles regarding how ethics should be approached in AI: 'fairness and non-discrimination', 'privacy, safety and security', 'human control of technology', 'transparency and explainability', 'accountability', 'promotion of human values', 'professional responsibility', and 'sustainable development'. These principles are further divided into three categories: 'avoiding undesired results', 'liability/acting responsibly', and 'ameliorating the lack of ethics in AI'. These categories and their respective principles will be explained further in this section. **Table 2** shows a breakdown of the three categories with a short description of each principle.

	Fairness and Non- Discrimination	AI algorithms that are non-discriminatory, fair, inclusive, representative, and free from human biases.
Avoiding Undesired	Privacy	AI use that enables consent, protection from surveillance, and right to control the use of the data gathered.
Results	Safety and Security	AI that does no harm to humans and resists external threats.
	Human Control of Technology	AI that remains under human control and enables review by those impacted.
Liability/Acting	Transparency and Explainability	AI that enables oversight and can be explained, understood, and recognized.
Responsibly	Accountability	Continuous assessment and evaluation of AI use, as well as the creation of new regulations and subsequent liability for failure to meet these regulations.
	Promotion of Human Values	AI that is used to benefit society, human civilization, and human rights.
Ameliorating the Lack of Ethics in AI	Professional Responsibility	AI that is designed purposefully and collaboratively with relevant stakeholders.
	Sustainable Development	AI that benefits or does not hinder the development of sustainable societies and objectives.

Table 2. Categorization of the nine principles of AI.

Avoiding Undesired Results

'Avoiding undesired results' is composed of "principles concerned with avoiding the dangers of AI when used for unethical or immoral purposes, whether intentionally or unintentionally".¹¹² The first principle in this category is 'fairness and non-discrimination'. Dubljevic's paper, Ethics of AI in Organizations, states that "care needs to be taken during both the creation and use of AI-based systems to ensure that human prejudice is not ingrained into the system before or after its initial deployment".¹ Fairness and non-discrimination' suggest that "AI should utilize only representative and high-quality data, be used impartially and equally across demographics, and consider a diverse array of stakeholders in its design and implementation".¹¹ The next principle is 'privacy'. It includes the right to consent to AI-based data collection, analysis, and measures of control over the subsequent use of the data.¹¹² After 'privacy', 'safety and security' are the next principles. 'Safety and security' propose that AI be protected from internal and external threats,¹¹⁰ as well as there be an element of predictability to the AI for the protection of society and individuals' safety.¹¹⁰ The last principle in this category is 'human control of technology'. Dubljević states that AI has to remain under human control and enable review by those the technology impacts.¹ The outcomes of AI are ultimately within human governance and "results and decisions stemming from AI technologies should be able to be reviewed, opted out of, challenged, or otherwise managed by people".¹¹⁰

Liability/Acting Responsibly

The next category, 'liability or acting responsibly', concludes that AI needs to be designed and utilized under appropriate scrutiny and within legal boundaries.¹ The first principle within this category is 'transparency and explainability'. This is defined by developing AI-based systems that may be easily managed and understood by experts and non-experts.¹ It can also be defined as AI that enables oversight and can be easily explained, understood, and recognized.¹0 The second principle categorized as liability and responsibility is 'accountability'. This refers to who or what is accountable for a decision made by an AI-based system and can be further divided into before, during, and after the use of the AI.¹ In Ethics of AI in Organizations, Dubljević states that "after an AI-based system's deployment, regulatory systems should exist to rectify unjust decisions made by the AI-based system, in addition to legal liability for those that cause harm using AI".¹

Ameliorating the Lack of Ethics in AI

After liability, the final category is 'ameliorating the lack of ethical values in AI'. The definition relating to this category is that AI is inherently amoral, therefore rules and laws are needed to guide the way that it is used, making sure it is ethical.^{1,11} One principle in this category is the 'promotion of human values'. The principle suggests that AI-based systems should be used for the common good and be deployed/developed consistent with human values.¹ AI systems should be widely available and distributed as equally as possible.^{1,10} Along with the equal distribution of AI systems, there needs to be a set of values that are agreed upon based on every culture and idea in the world. The most common values being considered include human dignity, human rights, and fundamental freedoms, leaving no one behind, living in harmony, trustworthiness, diversity and inclusiveness, and protection of

the environment.¹¹ The second principle in this category is 'professional responsibility'. It suggests that "AI be designed meticulously, purposefully, and with the input of stakeholders across a variety of levels".^{1,10} Designers of AI-based systems must consider the long-term effects of their creations, and must therefore ensure they are used in a reliable and valid manner.¹⁰ The final principle is 'sustainability/sustainable development', and it refers to "creating AI technologies that enable maintainable solutions to global problems such as healthcare and equality, minimizing resource waste, and environmental responsibility". ^{1,10} The Ethics of AI in Organizations, also states that it is important that the ethical principles of AI advocate for the avoidance of potentially disastrous outcomes of global warming.¹

AGENT-DEED-CONSEQUENCE (ADC) MODEL

The ADC Model predicts that moral judgment consists of three components: the character of a person (agent), their actions (deed), and the consequences of the situation (consequence). The ADC model implicitly applies the three moral theories to evaluate different aspects of a situation, as each component of the ADC model is tied to a moral theory. The model predicts that moral judgments are positive if all three of its components are considered positive, and negative if all three of its components are considered negative. This model is useful because it can help one categorize the ethical and unethical elements of a situation.

Ethical Theories	ADC Model
Virtue Ethics: Emphasizes the character of a person. It does not attempt to	Agent Component: Is related to virtue ethics, as it emphasizes the traits of
identify singular moral principles to any situation, rather, each person and	the person in a given situation.
situation should be evaluated individually.	
Deontology: Emphasizes whether the actions of a person are right or wrong,	Deed Component: Is related to deontology, as it emphasizes the actions
and whether those actions respect obligations, duties, and rights in a given	committed by a person and whether those actions follow moral principles.
situation.	
Consequentialism: Focuses on whether the outcomes of a situation are	Consequence Component: Is related to consequentialism, as it focuses on
morally correct or not. Related to utilitarian ethics which favors the option	the end results of a situation.
that will result in the most good	

Table 3. Ethical theories compared with the Agent-Deed-Consequence Model.

An example event will be explored to better understand each component of the ADC model. This scenario consists of emergency responders arriving at a collapsed building with people stuck under the debris. The emergency responders have a robot integrated with AI to assist them in deciding the most effective and efficient method for helping survivors out from underneath the rubble. The robot will analyze the conditions of the victims, assess the surrounding scenario, and make assumptions to determine how to act when coming across a victim. The victims would have to reach medical care as soon as possible, so the time for retrieval is critical.

Agent Component & Virtue Ethics

Virtue ethics emphasize the agency or character of a person, similar to the agent component of the ADC model.^{3,13} The theories of virtue ethics "do not aim primarily to identify universal principles that can be applied in any moral situation," unlike deontology and consequentialist theories, which do.⁴

In relation to the scenario, the Agent component in question would be the robot itself. Since the robot is proactively deciding who to save or who not to save based on specific circumstances, it acts as the individual who is in charge of making ethical decisions. The robot would utilize ethical virtues and the circumstantial information available to it in order to make the most appropriate decision.

Deed and Deontology

Deontology claims that an agent is ethical if "it respects obligations, duties, and rights related to given situations". Deontology specifically relates to the actions of a person, similar to the deed component of the ADC model. In the case of the example, the decision made by the robot to save certain people over others, or how the robot saved certain people, would make up the Deed component. A robot might decide to prioritize saving one person over another because it believes one has a much greater probability of survival. A robot might also decide that to save a person, it would have to cut off someone's leg if it is stuck under rubble and there is no quick way to remove it. The robot may have to be put in a position in which consent from the victim is "implied" if they are unconscious, and this would affect the ethical perception of human operators.

Consequence and Consequentialism

Consequentialism relates to the results of actions that are performed and defines virtues as traits that yield good consequences. It focuses on judging the moral worth of the results of actions, related to the consequences component of the ADC model. Regarding the scenario, the result of the assessment and decisions made by the robot would contribute to the Consequence component of the ADC model. If the people who were prioritized survived and those who weren't prioritized didn't, negative

backlash could arise against utilizing the robot. On the other hand, if the people the robot brought out of the rubble did not survive, a different negative backlash could occur due to the inability to save anyone.

CASE STUDIES OF AI FAILURES

While there has not been much research done on the ethical consequences of artificial intelligence, there are plenty of examples of failure of AI. In this section, case studies were conducted on three different AI failures to analyze how they violated ethical principles of AI. To prevent similar cases from occurring, a summary of the events, a discussion of the implications, and recommendations were given.

The three events reviewed in this section are an Amazon AI hiring tool, a company's AI system that carries out automatic tasks such as renewing contracts and access, and a Microsoft chatbot AI sent out on Twitter. These three AI systems failed to meet their expectations regardless of whether it was the programmers' fault or human input. In turn, there is something to be learned from each failure.

Case 1: The Sexist Amazon AI Hiring Tool

An AI recruiting tool from Amazon showed significant negative bias towards women when analyzing resumés. The experimental tool used an AI system to give candidates scores from one to five stars, based on their resumés, with the goal of selecting the top five applicants. The company soon realized that the AI tool was not selecting candidates for software development and other technical jobs gender-neutrally. Since the system was programmed to select resumés similar to those submitted to the company over a ten-year period, it inherited a bias towards men, as the company had mostly employed men in the past. Male dominance in the technological industry also played a role in this failure. The program took points away from resumés that included words referencing women and even "downgraded graduates of two all-women's colleges,". When trying to solve this problem, Amazon worked to edit the program, making the program remain neutral to terms referring to women but the system would continuously find a way to circumvent this, based on what it was originally taught.

Analysis

This particular case of AI mistake falls under the Ethics of AI category of 'Fairness and Non-Discrimination'. Dubliević states in his Ethics of AI in Organizations paper that "care needs to be taken during both the creation and use of AI-based systems to ensure that human prejudice is not ingrained into the system before or after its initial deployment". The 'fairness and nondiscrimination' principle, as detailed above, suggests that AI should only use highly representative data. Despite having a lack of diverse records, the AI system needs to consider that there are other participants in society outside of the data provided... The system needs to be able to consider the diverse demographics of the real world. In this case, the AI system did not obtain the original resources to have a highly representative set of data. The program was given the resumés that humans had gone through before which were biased as a result of the time period, i.e. not many women in the workforce or focused on the technological industry. Furthermore, Amazon's hiring tool did not promote human values by disregarding the common good of its applicants. Since the tool did not look at applicants objectively and give a fair chance to each one, based on sex, it did not promote the common good of each applicant. Amazon failed to follow two additional principles as well, 'professional responsibility and accountability'. Amazon declined to comment on the incident, ¹⁴ showing indifference and lack of accountability for the tool they implemented, declining applicants without human interaction. The refusal to comment on the incident also highlights a lack of diversity and misguided judgments on the implementation of ethical guidelines for this tool. This, in turn, opposes the principle of 'professional responsibility'. In reference to the three main ethical theories, deontology, virtue ethics, and consequentialism, the bias incident discussed, has a stake in each. Relating to deontology and action in the ADC model, in the beginning, an AI system should be created that is unbiased and fair in its selections and ideas. Taking the time to develop the program correctly and without bias will save time in the long run. The virtue ethics key to this issue is to create algorithms that are fair, inclusive, nondiscriminatory, and representative of all people; this is because the AI could be considered as an agent in making decisions, if the AI is treated as its own entity and considered capable of making its own decisions beyond what the developers had in mind. This is important because it not only makes places more diverse, but it shows a realistic portrait of the world right now. Finally, in reference to consequentialism, know the consequences of a biased program and the unfairness that comes from it. Everyone should be represented equally in a system. If there is fairness and non-discrimination in the development of the program, there will be less time spent trying to fix the system.

Recommendation

As companies are integrating AI into their systems and workplace, the indifference to consequences and possible errors associated with AI needs to be addressed. Using the Agent component of the ADC model, each applicant's character for the company should be analyzed. In relation to the Deed component of the ADC model, the action that is taken by the system should always be extensively researched before implementation and there should always be the ability to have human intervention. There should be a responsible party, making sure that the AI is following the correct guidelines, reevaluating each applicant based on their own

merits. The consequence component of the ADC model would be associated with the AI realizing that individuals should not be voted down when certain words are used in their resumé, especially when the initial sample of applicants was not representative of society as a whole. Through evaluating and implementing these components and characteristics of AI, a world will be created that promotes human values and equality.

Case 2: The Man fired by AI

Mr. Ibrahim Diallo became the victim of an AI failure when a system controlled by the company he worked for terminated his employment status with the company upon the expiration of his contract, without knowing of this expiration or termination. Diallo's false termination was first discussed after his key card would not allow him access to the building or the logins for his computer. Shortly after noticing, he seemed to have resolved the issue with his manager; nevertheless, security escorted him out of the building. Looking into the reason for his termination, Diallo found that the contract, originally signed when he was hired, had not been renewed. The AI system promptly terminated his employment at the company due to prior obligation and programming. This resulted in Mr. Ibrahim losing three full weeks of pay and the eventual resignation from his position. 15

Analysis

Diallo's story shows a need for ethics in AI and how automated, intelligent systems can result in harm not only to the employee but the employer as well. The company could lose effective employees through scenarios such as this one. This AI failure fits under the ethical principles of 'human control of technology', 'professional responsibility', 'transparency, and explainability', as well as 'privacy'. Humans remaining in control of technology connects with deontology as being able to review the impacts of technology and artificial intelligence. Without humans taking correct and decisive action against such failures, AI can continue to make these mistakes. Also, humans remaining in control of technology is aligned with consequentialism because if AI is not observed and inspected for mistakes by humans, there will be consequences in the future. Diallo's story connects with virtue ethics in that humans must continue to have 'professional responsibility' when it comes to AI. There needs to be a chain of command when it comes to the blame of AI, when it makes a mistake, especially in the professional world. It was the professional responsibility of Diallo's bosses to correctly reinstate his credentials within the computer system, instead of only reassuring him vocally that he had not lost his job. The lack of professionalism is eventually what made him have to leave his job. The company not taking the time to review Diallo's problem shows a lack of 'transparency and explainability'. It is the employer's responsibility to ensure the employee remains informed of their contract status. Nevertheless, the employers failed to do so. The privacy of Mr. Ibrahim was also breached when the company was required to escalate the situation to higher and higher levels of management. Diallo continuously had to ask others for their access to buildings and software because his had been revoked. This sharing of information not only hindered Diallo's privacy but the privacy of those around him. The consequentialist view of this situation is that artificial intelligence and other technology could get out of control and humans would not be able to stop it from making unethical decisions. The human view of the ethical decision-making process would possibly be obsolete.

Recommendation

Ibrahim Diallo's experience with his employer exacerbates the need for guidelines in reference to AI. His situation could have been avoided if the system prompted a human supervisor to approve of any major tasks/decisions such as completely revoking an employee's status with the company. The agent component of the ADC model connects to this AI failure by allowing for analysis and rechecking of decisions made by nonhumans. The deed component of the ADC model is that the system should have been double-checked during the affair as well as being equipped with a course of action for situations such as these. The employer should be able to stop the process of termination if need be. The consequence aspect of the AI failure is that Diallo's employer lost a good employee who was continuously regarded as "receiving constant praises" and whose "work spoke for itself," 16

Case 3: Microsoft Chatbot Learns Prejudice and Discrimination

Microsoft released a chatbot named "Tay" in 2016 with the intention of learning from humans on Twitter. The bot was meant to reflect the account of a teenage girl, learning from humans by interacting through tweets. In Just twenty-four hours after the chatbot was released, the tweets changed from, "humans are cool" to supporting neo-nazism, racism, misogyny, and genocide in its following interactions. Microsoft took down the chatbot swiftly and made an apology statement to Twitter users. Microsoft revealed that Tay was targeted by hate groups and Microsoft employees should've prevented this from happening.



Figure 1. Profile picture of chatbot Tay.

Analysis

Through the quick progression of Tay learning prejudice and discrimination, it is shown how human interactions can have negative effects on AI when not programmed keeping ethical considerations in mind. Tay was programmed to learn from interactions with users, without discerning between moral and immoral values, leading to failure in adhering to the principle of 'promotion of human values'.¹ Since the ethical principle proposes that AI should be used for the common good, Tay and other chatbots would need to be created with the capacity to differentiate between positive and negative human values, so that it may only learn the former. Tay is also an example of an AI that had an unintended and unpredictable outcome. Microsoft did not foresee Tay becoming discriminatory and prejudicial, this shows a level of unpredictability that breaks the ethical principles of 'fairness and non-discrimination' and 'safety and security'.¹ This major shortcoming of Tay shows how AI and machine learning, without proper safety nets and protocols, can lead to catastrophic events. Though Tay could not physically harm Twitter users, an AI that interacted with people personally in real-life applications could have had devastating effects on users and non-users alike. Take for instance an autonomous vehicle that could learn misogyny from its user. If a situation arose when a crash was inevitable between hitting a man or a woman, the AI may end up targeting the woman since the user demonstrated a negative bias towards women in general. If AI is created with the ability to learn characteristics about its user or users it interacts with, it must also be created with guidelines to not learn human notions such as misogyny, racism, or other hateful beliefs.

Recommendation

One method that can be employed to ensure AI only learns a certain set of values is through the use of the ADC Model. As the AI views and interacts with users, it has the opportunity to study their beliefs, attitudes, and behaviors to learn how to be better suited to users. Using the agent component of the ADC Model, the AI would have to recognize the intrinsic characteristics of the users it interacts with, as well as recognize how its agency may be viewed by others. Concerning the deed component, the AI would need to recognize actions that are inappropriate and ensure it does not copy them. In relation to the consequence component, the AI would need to realize how the results of its actions could impact others negatively. Through evaluating these characteristics, the AI would ensure its learned characteristics do not match negative human values and fulfill the three components of the ADC Model. Through AI only learning positive human virtues and values, AI will have the potential to benefit users and humanity as a whole.

Case	Principles	Overall Impact
The Sexist Amazon AI Hiring Tool	- Fairness and Non-Discrimination - Accountability - Professional Responsibility - Promotion of Human Values	AI needs to be created with the goal of treating everyone equally no matter the gender, race, ethnic background, sexual orientation, etc.
The Man Fired by AI	- Transparency and Explainability - Human Control of Technology - Professional Responsibility - Privacy	AI systems may need human input before carrying out major tasks.
Microsoft Chatbot Learns Prejudice and Discrimination	- Fairness and Non-Discrimination - Safety and Security - Promotion of Human Values	AI must not inherit human biases that can harm humans or other AI. Additionally, it is important for humans to realize the impact they have on AI intelligence and learning.

Table 4. Table analyzing each of the cases of AI failures and their overall impact on society.

REVISED PRINCIPLES & DEVELOPED MODEL

As was the intention from the beginning, a model was created to help identify the unethical aspects of a situation in which AI failed or committed failures. Through the analyses of three unethical AI failures, a model was developed that would help guide society to recognize specific unethical aspects of AI. Going through the steps in the model, one can determine what components of the ADC model were unethical, what principles of ethical AI were violated, as well as how AI was involved in the failure. The model is named the "AI Failure Analysis Model (AIFAM).

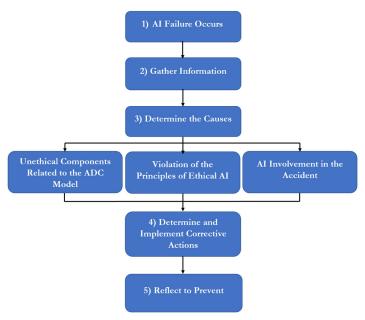


Figure 2. The AI Failure Analysis Model (AIFAM).

It is recognized that this model could be useful for creators or users of AI to look back and ensure their systems operate ethically after a failure occurs. The model could be utilized at companies such as Tesla, Amazon, or Google to objectively reevaluate their AI, and make necessary changes to it if necessary. Using the model, companies would demonstrate, to the public, how they plan on identifying and repurposing their AI after a failure occurs.

Steps of the Model

In this subsection, the steps of the AIFAM model are described and explained.

- 1. AI Failure Occurs
 - a. AI fails and does not fulfill its purpose; the AI deviates from its programmed intention.
- 2. Gather Information
 - a. The person/group conducting research on the failure obtains as much information about the failure, the AI used, and any helpful contextual information.
- 3. Determine the Causes
 - a. At this step, the factors that led to the failure are examined. Each component has sample questions to help identify the causes and unethical aspects of the failure.
 - b. ADC Model:
 - i. What aspects of the situation were unethical? Agent, Deed, and/or Consequence?
 - ii. How were they unethical?
 - iii. Why were they unethical?
 - c. Violation of the Principles of Ethical AI
 - i. What principles of ethical AI were not followed?
 - ii. How were they not followed?
 - iii. Why were they not followed?
 - d. AI Involvement in the Failure
 - i. What was the AI originally intended/programmed to do?
 - ii. How did the AI fail to meet its programming?
 - iii. Why did the AI not follow its protocols?
- 4. Determine and Implement Corrective Action
 - a. By knowing the causes for which the AI acted unethically, it is possible to determine how to tackle those causes to ensure they do not cause more failures. One should ensure these failures are not carried out by implementing corrective actions on the AI.
- 5. Reflect to Prevent
 - a. Using the information collected, the analysis made, and the corrective action determined and implemented, one is able to reflect on the AI failure as a whole and ensure similar occurrences do not occur in the future.

DISCUSSION & CONCLUSION

The different failures involving AI showcase how ethical principles can be infringed upon in real-world applications. Though the failures of imperfect AI pale in comparison to science-fiction movies such as *The Terminator*, where the AI evolves on its own and attempts to carry out human extinction, the effects of these failures were still felt by real people such as Diallo who resigned his position and women who could have been hired by Amazon. The creators of AI must adopt ethical mindsets if they hope for their systems to operate ethically and have little to no possibility of harming human beings. Ethical principles are designed to protect humans from the dangers of AI, and they are further used to advance humanity as a whole to greater heights. Specific instructions on how to program or teach AI to respect and adhere to the ethical principles of AI are not provided, as that is outside the scope of this paper. However, guidance is given to ensure AI remains ethical in its growth and development.

Through exploring widely different examples of failures in AI, ethical principles of AI were observed in real-world applications. The Amazon AI hiring system trespassed on the principle of fairness and non-discrimination. The AI wrongfully firing a man violated privacy and professional responsibility. Microsoft's Tay chatbot could not fulfill the promotion of human values. These failures show why ethics in AI is important, and they encourage the creators of AI to prevent these failures from occurring again by taking closer looks at the causes of the events and why the AI might've acted the way it did. The AIFAM was created to analyze these events, and prevent them from happening again in the future, as it incorporated information from the ADC model, the principles of ethical AI, and the AI failure itself.

Nevertheless, the AIFAM should not be seen as a definitive and final guide to analyzing past failures and preventing future failures. Rather, it should be regarded as a starting point for ensuring AI remains ethical as it develops throughout the years. Future research is needed in areas such as analyzing more specific details of why AI behaves the way it does and how certain acts could be viewed as ethical or not to AI. These two topics of research may enhance the AIFAM or may need their own model to more closely examine the causes or why AI acts unethically. Having the AIFAM serve as a starting point for more research, it is hoped that AI remains ethical in terms of both its use and development.

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REFERENCES

- 1. Dubljević, V. (2022) Ethics of AI in Organizations in Human-Centered Artificial Intelligence (Nam, C; Jung, J; Lee, S., Ed.) 221-239, Elsevier. DOI:10.1016/B978-0-323-85648-5.00019-0
- 2. Ouchchy, L., Coin, A. & Dubljević, V. (2020) AI in the headlines: The portrayal of the ethical issues of artificial intelligence in the media. AI & Society, Volume 35, 927–936. https://doi.org/10.1007/s00146-020-00965-5
- 3. Athanassoulis, N. (2007) Virtue Ethics, Internet Encyclopedia of Philosophy: A Peer-Reviewed Academic Resource, https://iep.utm.edu/virtue/
- **4.** Statman, D., Trianosky, G.V. (1997) What is Virtue Ethics All About?, *Virtue Ethics* (Cambridge: Edinburgh University Press)
- Dillon, J. (2021) Utilitarian vs deontological ethics in medicine and dentistry, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, Volume 132, Issue 6, 617-618, https://doi.org/10.1016/j.oooo.2021.08.025.
- **6.** Dubljević, V., and Racine, E. (2014) The ADC of moral judgment: Opening the black box of moral intuitions with heuristics about agents, deeds and consequences. *AJOB Neuroscience*, Volume 5, Issue 4, 3–20.
- 7. Dewey, J. (1966): Three independent factors in morals. Educ Theory.; Volume 16:198–209.
- Yu, H., Shen, Z., Miao, C., Leung, C., Lesser, V. R., & Yang, Q. (2018). Building ethics into Artificial Intelligence. Proceedings of the Twenty-Seventh International Joint Conference on Artificial Intelligence (IJCAI 2018), 5527–5533. https://arxiv.org/abs/1812.02953v1
- 9. Grincevičienė, V., Barevičiūtė, J., Asakavičiūtė, V., & Targamadzė, V. (2019) Equal opportunities and dignity as values in the perspective of I. Kant's deontological ethics: The case of inclusive education. Filosifija Sociologija., Volume 30, Issue 1, 80-88. https://www.proquest.com/scholarly-journals/equal-opportunities-dignity-as-values-perspective/docview/2213858860/se-2?accountid=12725
- **10.** Fjeld, J., Achten, N., Hiligoss, H., Nagy, A. C., & Srikumar, M. (2020) Principled Artificial intelligence: Mapping consensus in ethical and rights-based approaches to principles for AI. Berkman Klein Center for Internet & Society. https://dash.harvard.edu/handle/1/42160420
- 11. UNESCO, (2020) Elaboration of a Recommendation on the ethics of artificial intelligence, Ad Hoc Expert Group, https://en.unesco.org/artificial-intelligence/ethics

- **12.** Dubljević, V., Sattler, S., & Racine E. (2018) Deciphering moral intuition: How agents, deeds and consequences influence moral judgment, *PLOS One*, Volume 13, Issue 10, https://doi.org/10.1371/journal.pone.0204631.
- **13.** Zizzo, N., Bell, E., & Racine, E. (2016) What Is Everyday Ethics? A Review and a Proposal for an Integrative Concept. *The Journal of Clinical Ethics. https://pubmed.ncbi.nlm.nih.gov/27333062/*
- **14.** Dastin, J., (2018) Amazon scraps secret AI recruiting tool that showed bias against women, Reuters, https://nnw.reuters.com/article/us-amazon-com-jobs-automation-insight-idUSKCN1MK08G
- 15. Wakefield, J. (2018) The man who was fired by a machine. BBC News. https://www.bbc.com/news/technology-44561838
- 16. Diallo, I. (2018) The Machine Fired Me. iDiallo. https://idiallo.com/blog/when-a-machine-fired-me
- Wakefield, J. (2016) Microsoft chatbot is taught to swear on Twitter. BBC News. https://www.bbc.com/news/technology-35890188.
- **18.** Reese, H. (2016) Why Microsoft's 'Tay' Ai Bot Went wrong. TechRepublic. https://www.techrepublic.com/article/why-microsofts-tay-ai-bot-went-wrong/.
- BBC. (2018) Amazon scrapped 'sexist AI' tool. BBC News. https://www.bbc.com/news/technology 45809919.
- 20. Lee, P. (2016) Learning from Tay's introduction. The Official Microsoft Blog. https://blogs.microsoft.com/blog/2016/03/25/learning-tays-introduction/#sm.00000gjdpwwcfcus11t6oo6dw79gw.
- 21. Price, R. (2016) Microsoft's genocidal AI chatbot is broken again. Business Insider. https://www.businessinsider.com/microsoft-ai-tay-twitter-racist-genocidal-breaks-down-repeats-too-fast-2016-3.
- 22. United States National Transportation Safety Board (1995) Marine Accident Report: Grounding of the Panamanian Passenger Ship Royal Majesty on Rose and Crown Shoal Near Nantucket, Massachusetts June 10, 1995. https://permanent.fdlp.gov/websites/www.ntsb.gov/publictn/1997/MAR9701.pdf
- 23. Wallach, W., Allen, C. & Smit, I. (2008) Machine morality: bottom-up and top-down approaches for modelling human moral faculties. *AI & Society*, Volume 22, 565–582. https://doi.org/10.1007/s00146-007-0099-0
- 24. Andersen, S. (2001) Theological Ethics, Moral Philosophy, and Natural Law. Ethical Theory and Moral Practice, Volume 4, 349–364. https://doi.org/10.1023/A:1013318824823
- 25. Knoll, M. (2019). Machiavelli's Consequentialist Ethics of Responsibility. History of Political Thought, Volume 40, Issue 4, 631-648. https://proxying.lib.ncsu.edu/index.php/login?url=https://www-proquest-com.prox.lib.ncsu.edu/scholarly-journals/machiavellis-consequentialist-ethics/docview/2312965948/se-2
- **26.** Stanziani, A. (2021). Utilitarianism and the question of free labor in russia and india, in the eighteenth and nineteenth centuries. *International Journal of Asian Studies*, Volume 18, Issue 2, 153-171. *doi: https://doi-org.prox.lib.ncsu.edu/10.1017/S1479591420000583*

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PRESS SUMMARY

Have you wondered how Artificial Intelligence (AI) makes decisions? What about the ethics of those decisions? How does AI help or harm different people? Artificial intelligence is not always perfect and is known to cause failures that impact a multitude of people. The purpose of this study is to explore how ethical guidelines are followed by AI when it is being designed and implemented in society. Three ethics theories, along with nine ethical principles of AI, and another model associated with ethics were investigated to analyze failures involving AI. When a system fails to follow the models and theories, a set of refined ethical principles are created. By analyzing these AI failures, an understanding of how similar incidents may be prevented can be gained. Additionally, the importance of ethics being a part of AI programming is demonstrated, followed by recommendations for the future incorporation of ethics into AI.

The By-Product of Ozone from Electrostatic Air Cleaners

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ABSTRACT

Indoor Air Quality (IAQ) contributes to the health and comfort of people living and working indoors. Poor IAQ can be linked to indoor and outdoor sources of contaminants. One recent solution for improving IAQ is the use of Electrostatic (ES) air cleaning technology. An ES air cleaner can be installed in a heating, ventilation, and air conditioning system where it pre-filters large dust particles and shocks smaller particles into a collection tray. However, ES air cleaners have been known to give off ozone as a byproduct, which is, itself, an air contaminant. Ozone is found outdoors as product of sunlight combining nitrogen oxides and volatile organic compounds generated from man-made pollution. Indoor ozone concentration will depend on the introduction of outdoor ozone indoors through natural ventilation, mechanical ventilation, and infiltration through the building's envelope (in order of importance). Two different ES air cleaners, A and B, were installed in the air conditioning system of research house #2 of the TRANE Residential Heating and Cooling Research Lab at the University of Texas at Tyler. A series of ozone experiments were conducted, which included measuring the baseline ozone levels at the research houses with different levels of insulation, observing the increase in ozone due to the powering on of mechanical ventilation, and observing the increase in ozone due to the powering on of the installed ES air cleaners. The baseline ozone levels observed in research house #2, whose envelope is more tightly insulated, was found to be lower than in research house #1 whose envelope is less tightly insulated. With regards to mechanical ventilation, an increase in ozone levels were seen in addition to an even higher increase in ozone levels when the ES air cleaners were powered on in tandem. In terms of the single contribution of the ES air cleaners in raising indoor ozone levels, the data shows that although the ES air cleaners increased the ozone concentration in the house, the levels are not of concern as they were less than the FDA limit on indoor ozone generation.

KEYWORDS

Indoor Air Quality, Ozone; Electrostatic Air Cleaner; Infiltration; Mechanical Ventilation; HVAC; Pollutant; Indoor Contaminant

INTRODUCTION

Indoor Air Quality (IAQ) refers to the quality of air within and around buildings and structures.¹ IAQ contributes to the health and comfort of people living and working indoors where most of our lives, around 90%, are spent. Indoor concentrations of pollutants can be up to two to five times the concentration found outdoors, depending on indoor sources, which can lead to lack of productivity and negative health effects for indoor residents.² The primary causes for a deficiency in IAQ include indoor sources of air pollutants, and the infiltration of outdoor pollution. Outdoor air contamination enters a residential house through cracks and openings in the house's envelope. The most common indoor pollutants include fine particulate matter (PM), volatile organic compounds (VOCs), and carbon dioxide (CO₂); in addition to ozone (O₃), which is the contaminant of consideration in this study.

One solution for improving IAQ, in the abatement of indoor contaminants, have been air cleaners. There are different technologies for air cleaners such as photocatalytic oxidizers (PCO) air cleaners and electrostatic (ES) air cleaners, etc. However, even though air cleaners assist in reducing contaminants, some are known to give by-products of another contaminant. Electrostatic air cleaners work to reduce PM in an indoor environment; however, they are known to produce ozone as a by-product. They are sold as portable air cleaners placed in singular locations and air cleaners installed in-duct that work to clean the air in all rooms integrated in the heating, ventilation, and air conditioning (HVAC) system.

Ozone is a highly reactive gas, composed of three oxygen atoms. At the stratospheric level, the earth's upper atmosphere, ozone is formed through the interaction of solar radiation with molecular oxygen. Stratospheric ozone is essential as it protects the surface of the earth from harmful ultraviolet (UV) radiation from the sun. Tropospheric ozone is harmful because of its proximity to humans at ground or "breathing" level as an air contaminant. The EPA has an air quality index (AQI) related to 8-hour averages of ozone concentrations separated in categories classified as good (0-54 ppb), moderate (55-70 ppb), unhealthy for sensitive groups

(71-85 ppb), unhealthy (86-105 ppb), very unhealthy (106-200 ppb), and hazardous (201+ ppb). The EPA also states that a 2-hour average of 600 ppb ozone concentration is considered a significant harmful level with imminent effects. Tropospheric ozone is considered the outdoor ozone that surrounds buildings and structures. Outdoor ozone is found outdoors as product of sunlight combining nitrogen oxide (NO_x) and VOCs generated from automobiles and coal-fired power plants. In addition to VOCs generated from trees and vegetation. This leads to urban areas having higher levels of outdoor ozone than rural areas where there is less pollution. This is also why outdoor ozone is higher in the daytime and, seasonally, in the summertime where there is more sunlight in comparison to wintertime. Geographical location and meteorological conditions are also factors in outdoor ozone concentrations in terms of the production and transport of outdoor ozone.

Indoor ozone concentration will depend on the introduction of outdoor ozone indoors through natural or mechanical ventilation, and infiltration through the envelope. Indoor ozone concentrations will also depend on known indoor sources of ozone by some electrical devices that give off ozone as by-products. Such as electrostatic (ES) air cleaners, photocopiers, laser printers, *etc.*⁷ FDA standard (21CFR801) is related to products that emit ozone as a by-product. The standard states that devices that generate ozone as a by-product should not generate ozone in excess of 50 ppb or parts-per-billion. This concentration is related to both the volume of air circulating through the device or an accumulation of ozone in an enclosed space intended to be occupied by humans. Enclosed spaces include houses, apartments, hospitals, and offices.⁸

This paper focuses on the contribution that two in-duct ES air cleaners, A and B, had in raising indoor ozone concentrations. This paper also presents literature on meteorological factors that influence outdoor ozone, and the effect of envelope characteristics on the indoor infiltration of outdoor ozone. Two ES air cleaners, identified as A and B to avoid mentioning brands, were evaluated for their contributions in raising the indoor ozone concentration at one of the two test and research houses at the University of Texas at Tyler. Additional ozone experiments were run to read baseline indoor ozone levels, indoor ozone levels with the introduction of mechanical ventilation, and a comparison of indoor ozone concentrations between the two houses that have different construction characteristics.

Characterization of the tropospheric boundary layer and outdoor ozone

The earth's surface layer is a part of the tropospheric boundary level (BL) which is the lower-level atmosphere that is affected by the friction and transfer of heat from the earth's surface. The tropospheric BL develops periodically throughout the day. In the morning, sunlight heats the earth's surface creating a transfer of heat into the atmosphere; eroding the stable layer formed overnight through radiational cooling. The new BL combines surface heating and wind turbulence and creates what is known as the mixed layer, reaching its maximum depth in the afternoon. The rapid growth of the mixed layer coincides with the mixture of outdoor pollutants. As the sun sets, and solar radiation decreases, a new stable nocturnal BL is established which leads to the discontinuity of pollutants at this level. However, there is a residual layer at a higher elevation that contains pollutants from the daytime. An example of the diurnal cycle can be found in *Javob, J. D.*¹⁰

Ozone is produced photochemically by the oxidation of methane, carbon monoxide (CO), and non-methane hydrocarbons (NMHCs) in the presence of nitrogen oxides and sunlight. This process leads to ozone having strong diurnals variations near the earth's surface layer. During the afternoon, mixing ratios of outdoor ozone increase at its highest coinciding with the combined effect of photochemical production and the mixing of ozone rich air masses from the residual BL. At nighttime, mixing ratios of outdoor ozone are at its minimum due to dry deposition and a lack of solar radiation in the shallow nocturnal BL. Dry deposition is when particles are removed from the atmosphere due to gravity. ¹²

Diurnal and seasonal characteristics of outdoor ozone

The diurnal and seasonal cycle of outdoor ozone can be related, generally, to solar radiation as stated in the *introduction*. This correlation is illustrated by a study that was conducted by the Department of Environmental Sciences at King Abdulaziz University (KAU) which observed the diurnal and seasonal variations in Yanbu, Saudi Arabia. **Table 1** shows the minimum and maximum outdoor VOC concentrations seen in a diurnal cycle for four seasons, respectively.¹³

	Winter	Spring	Summer	Autumn
Maximum VOC concentration (ppb) / Hour of occurrence	28/17.5	39/12.5	36/11.5	32/14.5
Minimum VOC concentration (ppb) / Hour of occurrence	8/8.5	12/7.5	13/7.5	10/7.5

Table 1. Maximum and minimum VOC concentrations seen in a diurnal cycle for four seasons.¹³

A couple of things can be concluded from **Table 1**. One is related to the diurnal cycle of outdoor ozone. The minimum concentrations of ozone occurred at early hours (7:30 or 8:30 AM) for every season. The maximum concentrations of ozone

occurred at later hours (11:30 AM, 12:30 PM, 2:20 PM, or 5:30 PM) for every season. This is consistent with the characteristics of the diurnal cycle of outdoor ozone, showed to follow the development of the tropospheric BL mentioned in the *Characterization of the Tropospheric Boundary Layer and Outdoor Ozone* section. Outdoor ozone from the residual BL is further mixed and created as solar radiation increases throughout the day. Outdoor ozone levels reach their peak at the height of sunlight. As the sun sets, and solar radiation decreases, outdoor ozone concentrations begin to diminish and stabilize into the nocturnal BL. Another conclusion from **Table 1** is that outdoor ozone has a seasonal cycle. The highest range of outdoor ozone concentrations is seen in the spring followed by summer, autumn, and is lowest in the winter. A ranking of months which can also be related to the amount of solar radiation seen in those months.

Trends of outdoor ozone in areas of different urbanization

For the most part, outdoor ozone levels are higher in urban areas than rural areas. Higher levels of nitrogen oxides and man-made VOCs are found in urbanized areas which combine under sunlight to produce ozone. This process is as follows and is shown in **Equations 1 through 3**. Nitric oxide (NO) from the oxides of nitrogen (emitted mainly from fossil fuel combustion) mix with existing ozone (O₃) into nitrogen dioxide (NO₂) and oxygen (O₂). The nitrogen dioxide is later photo-dissociated (hv) back into nitric oxide and a singular oxygen atom. The singular oxygen atom then combines with oxygen compounds to form ozone with a third stabilizing molecule (*M*) that works to remove excess energy.¹⁴

$$NO + O_3 \xrightarrow{\text{yields}} NO_2 + O_2$$
 Equation 1.

$$NO_2 + hv \xrightarrow{yields} NO + O$$
 Equation 2.

$$O_2 + O + M \xrightarrow{yields} O_3 + M$$
 Equation 3.

The Greek research article "Indoor Air Pollution: The Case of Ozone in Three Regions in Greece" included the dependence of outdoor to indoor ozone concentration ratios on the degree of urbanization. A full diurnal cycle of data was taken from the metropolitan city of Athens and the less-urbanized Zakynthos Island. Outdoor ozone levels peak much higher in Athens than Zakynthos island. The maximum outdoor ozone concentration seen in Athens was 90 ppb and 55 ppb in Zakynthos. Higher levels of outdoor ozone translate to higher indoor levels of ozone as well. The maximum indoor concentrations of ozone seen in Athens was 55 ppb and 25 ppb in Zakynthos Island. The comparison of the two cities illustrates how more urbanized areas with more pollution contribute to higher levels of outdoor ozone which translate to higher indoor levels of ozone. In is important to state that high outdoor ozone concentrations can be seen in rural areas due to the transportation of ozone which will discussed in the following section. In the following section.

The effect of wind on the transportation of outdoor ozone

Ozone can be transported from major cities into rural areas with the downwind of ozone and its precursors (NO_x and VOCs). Outdoor ozone levels in urban areas peak during afternoon hours at the height of sunlight intensity. Rural areas where outdoor ozone levels are high, usually peaks in outdoor ozone levels in the late afternoon or evening from the ozone transported from more urbanized areas. Depending on wind speeds and patterns, outdoor ozone levels can be transported hundreds of miles downwind. There is a map in **EPA** that shows a map of the US with four colors representing four ranges in outdoor ozone concentration. The darker colors highlight areas which have higher levels of outdoor ozone. For the most part, the darker colors are seen in areas with major cities that are highly urbanized. However, the rural areas around the urbanized areas experience heightened levels of outdoor ozone. Just at lower concentrations. This effect can be linked to wind patterns. The Commission for Environmental Cooperation (CEC) has a report on the long-range transport of ground level ozone and its pre-cursors. The report states the regional extent of high outdoor ozone concentrations from urbanized regions with high pre-cursor emissions is highly dependent on wind patterns. During low-speed wind cycles, heightened ozone levels tend to remain near the region of formation¹⁴.

A study named "Influence of local meteorology and NO₂ conditions on ground-level ozone concentrations in the eastern part of Texas, USA" included an analysis on the effect that wind speed, wind direction, and nitrogen dioxide had in the concentrations of outdoor ozone. The study collected data from various monitoring stations in east Texas counties. In the correlation analysis, it was found that increases in nitrogen dioxide levels led to higher levels of outdoor ozone. The two variables had positive correlation coefficients through the course of a week in May. The coefficients were significant at a significance level of 1%. Variations were seen in the locations of maximum ozone with respect to time, even though the maximum concentrations of nitric dioxide were measured near the region of formation. Which leads back to the principle of the transportation of ozone due to wind.

The study observed the transportation of high outdoor ozone concentrations from regions of high nitric dioxide levels based off wind speed and direction. Seven days were chosen for observation with the help of spatial distribution maps of ozone, which were used to identify the movement of high outdoor ozone levels. On days that significant wind patterns were observed, high levels of outdoor ozone were observed in areas around the regions of formation. These areas were found to be in the direction of wind moving from the regions of formation. On the days that wind speeds intensified; high outdoor ozone levels were transported to a greater extent in the direction of the prevailing winds. On days that the wind speed were not as strong, no significant transportation of ozone was seen. The study concluded that the distribution patterns of outdoor ozone were definitely influenced by wind speed and direction.¹⁷

Infiltration of outdoor ozone into an indoor environment

Outdoor ozone can infiltrate a building's envelope in three different ways. Through cracks and openings in the exterior, natural ventilation, and mechanical ventilation. A parameter used for measuring the infiltration of outdoor in an indoor environment is the I/O ratio. The I/O ratio relates the concentration of indoor ozone as a percentage of outdoor ozone concentrations. Each of the three paths of infiltration have different I/O ratios. Differences in I/O ratios between the two types of ventilation are due to the fact that outdoor ozone will pass through more filtering in mechanical ventilation than natural ventilation. Infiltrations through cracks and exterior opening have the smallest effect in raising the I/O ratio and will depend on the tightness of the building's envelope.

A study called "Study of outdoor ozone penetration into buildings through ventilation and infiltration" observed I/O ratios under the three paths of outdoor ozone infiltration. The study included the summary of a literature review on I/O ratio values seen in 385 houses for the three paths of infiltration. I/O ratios were listed as 0.09, 0.19, and 0.47 for infiltration, mechanical ventilation, and natural ventilation respectively. These values corresponded to median air exchange rates, or air changes per hour (ACH), and surface deposition rates. The study concludes listing the paths of infiltration in order from lowest to highest I/O ratios. This means that natural ventilation led to the biggest infiltration of outdoor ozone indoors, followed by mechanical ventilation, and infiltrations through cracks.¹⁸

Another study investigated, "The impacts of building envelope design on indoor ozone and health exposures in residential houses". Data was recorded in four rooms, from three houses with old construction, with different exterior finishes and levels of tightness. None of the houses had any significant sources of indoor ozone such as photocopiers or printers. The houses were occupied with no restriction on their daily routines, and data was taken over the period of 10 days. The constructions of the four envelopes with their respective I/O ratios are summarized in **Table 2**.

Envelope	Exterior Wall Finish	Wall surface area (ft²)	Window Perimeter (ft)	Window to Wall Ratio	Wall Thickness (in)	I/O ratios
1	Stucco	143	33	0.21	11.0	0.49 ± 0.24
2	Brick	134	33	0.22	16.0	0.52 ± 0.18
3	Brick	176	61	0.30	12.5	0.68 ± 0.19
4	Painted fiber cement siding	79	31	0.95	9.8	0.48 ± 0.2

Table 2. Construction of envelopes 1, 2, 3, and 4.19

The factors of the envelopes were statistically analyzed and correlated with indoor ozone concentrations. The study found that the envelope construction variables that played important roles in influencing indoor ozone concentrations were exterior wall finishing and window to wall ratio. Exterior materials can chemically react with outdoor ozone and diffuse ozone before it penetrates indoors. The study reveals mixed effects from the window to wall ratio. The study concluded that the construction variables observed in the study are reasonable predictors of indoor ozone levels.

Generation of ozone from ES air cleaners

In-duct ES air cleaners, installed in HVAC systems, are manufactured to remove a wide range of airborne particles. The standard operating procedure for ES air cleaners consists of three parts which include ionization, collection, and filtering. ES air cleaners first ionize incoming contaminant particles by generating a field of static electricity. The particles are then collected in a series of discharge plates with laminated film envelopes which are separated by a small intermediate distance. The film is a high dielectric material used as an electrical barrier to prevent electric sparks from an electrical discharge. Carbon filters, positioned as the final step in ES air cleaners, are filters that contain granular pieces of carbon. Remaining contaminated particles react chemically with the carbon material and stick to the filter. Thus, preventing the particles from recirculating back into the house. The ionization of the contaminant particles also leads the ionization of oxygen passing through the ES air cleaner. The formation of ozone through ionization can be simplified in a two-step process as seen in **Equations 4 and 5** where M is a third stabilizing molecule (M).²²

$$e + O_2 \xrightarrow{yields} 2O + e$$
 Equation 4.
 $O + O_2 + M \xrightarrow{yields} O_3 + M$ Equation 5.

There is a literature review paper named "Electrostatic Precipitators as an Indoor Air Cleaner—A Literature Review" which summarized publications on ES air cleaners. The paper summarizes aspects related to ES air cleaners design that lead to higher generation rates of ozone, methods of ES air cleaner testing, and results to the extent that in-duct ES air cleaners had in raising indoor ozone levels in a manufactured test house. The extent to which ozone is generated can be related to product design and operating conditions of the ES air cleaner. Some design factors include charging wire diameter and material, geometry of the Es air cleaner, and the applied voltage. With respect to operating conditions of the ES air cleaner, a higher operating setting could lead up to a 50% increase in ozone generation as opposed to a lower setting.²³ A poorly designed ES air cleaner could raise indoor ozone levels above the recommended limit of 50 ppb. For this reason, there are standards given by the California Air Resources Board (CARB) and UL (an accredited standards developer) that are used to certify ES air cleaners in terms of ozone generation with approved test methods.²⁴ The literature review paper mentioned a study that found the use of the two studied ES air cleaners raised indoors ozone levels by 77 and 20 ppb, respectively, with the study concluding that the largest influence in ozone production was the brand of the respective ES air cleaner.²⁵

Another study, "Characterization of potential indoor sources of ozone", included the observation of indoor ozone levels in homes with permanently installed ES air cleaners. The study first measured indoor background ozone levels downstream of the air cleaner, powered off. A total of eight in-duct ES air cleaners were evaluated. The blower ran continuously. Then the ES air cleaner was powered on, and downstream ozone was measured. The study decided to measure ozone downstream and near supply registers to measure the maximum possible ozone before dissipating into the house. Two of the measured air cleaners produced ozone. However, these measurable quantities of ozone decayed to non-delectable levels as it passed through the ventilation ducts. The study concludes by stating that the ozone emission rates of the ES air cleaners would not produce concentrations greater than 10 to 30 ppb above background indoor ozone levels.²⁶

MATERIALS AND METHODS

The ozone experiments were conducted at the two test and research houses at the University of Texas at Tyler. The test houses are identical in size and layout with an area schedule of 1,470 square feet (excluding the garage, covered porch, and covered patio). The houses have an interior volume of 11,939 cubic feet and are shown in **Figure 1**. Test house #2 (on the left) has a tight building envelope, while test house #1 (on the right) is draftier. They have a brick exterior finish with a total of nine windows for each house. Neither test houses have regular occupants, nor are they furnished.



Figure 1. Test houses at the University of Texas at Tyler.

All experiments included the fan being powered on continuously at 100% allowing for good air mixing within the test house at 4.03 air changes per hour (ACH). Experiment 1 was set to show baseline indoor ozone levels (due to infiltrations only) in house #2. Experiment 2 compared the baseline indoor ozone concentrations of the test and research houses. Experiment 3 highlighted the increase in indoor ozone from experiment 1 due to ventilation, and in experiment 4, the ES air cleaner is powered on. In experiment 5, ventilation was powered off to show the increase in indoor ozone due only to the powering on of an ES air cleaner.

Experimental 6 is identical to experiment 5 with the only difference being that indoor ozone concentrations were measured near supply registers. Experiments 3 through 5 were repeated for both ES air cleaners A and B. Both ES air cleaners were set to their respective max on setting. It important to note that ES air cleaner A had an actual distinction between on and max-on, while ES air cleaner B only had an on option.

The Model 202 ozone monitor from 2B Technologies was used to monitor ozone in the living room. The ozone monitor was zeroed out for every experiment (see Appendix A). Readings from the ozone monitor have an uncertainty of ±1 ppb or 2% of the reading. Since all readings inside the house are below 50 ppb, the uncertainty associate to reading inside the house is 1 ppb. The ozone monitor was stationed inside the test house and placed in two different positions throughout all the ozone experiments. For ozone experiments 1 through 4, the ozone monitor was placed on a stool underneath the supply registers as illustrated in **Figure 2**. In ozone experiment 5, the ozone monitor was placed on a ladder about an inch and a half from the ceiling close to the supply registers as illustrated in **Figure 3**. The location of the stool setup was determined as the result of a stratification test and a test that measured the critical point of ozone in the test house (see Appendix B).





Figure 2. Stool setup for the ozone monitor.





Figure 3. Ladder setup for the ozone monitor.

Figure 4 shows the location of the ozone monitor and the four nearest supply registers with respect to the house layout.

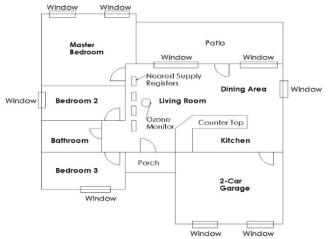


Figure 4. Ozone monitor location and nearest supply registers in house layout.

Table 3 summarizes the purpose of all six ozone experiments.

Experiment	Description	Duration (Days)
1	Baseline indoor ozone levels (due to infiltrations only) in house #2	1
2	Baseline indoor ozone concentrations of both test houses	1
3	Increase in indoor ozone from baseline ozone levels due to mechanical ventilation	ES air cleaner A: 1 ES air cleaner B: 1
4	Indoor ozone levels with ES air cleaner powered on and with mechanical ventilation	ES air cleaner A: 1 ES air cleaner B: 1
5	Indoor ozone levels with ES air cleaner powered on	ES air cleaner A: 2 ES air cleaner B: 3
6	Indoor ozone levels with ES air cleaner powered on (measured at the supply register)	ES air cleaner A: 2 ES air cleaner B: 3

Table 3. Description of ozone experiments.

RESULTS AND DISCUSSION

For all plots in the results section, the horizontal green line represents the FDA standard 21CFR801 mentioned in the *introduction* and the vertical red line separates days of data.

Baseline indoor ozone levels in UTT house #2

Figure 5 shows the ozone data for ozone experiment 1, which had a duration of 48 hours.

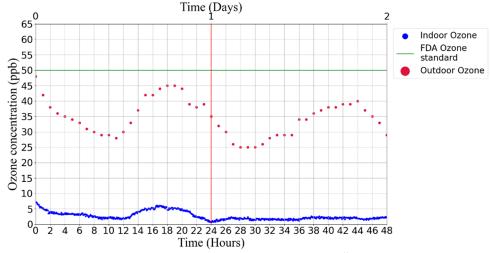


Figure 5. Ozone experiment 1 results for house #2.

Since there is no source of ozone inside the house with the electrostatic filter is off, and no ventilation, levels of ozone can only be affected by infiltrations in house #2. As discussed in the *Infiltration of Outdoor Ozone into an indoors environment* section, I/O ratios tend to be affected the least due to infiltrations and will depend on the tightness of the building's envelope. This seems to be the case from the data in **Figure 5**. The indoor ozone levels are following the outdoor trend at a very low magnitude. This is representative of the fact that house #2 is constructed with a tight envelope. The average baseline concentration of indoor ozone seen in the house is 2.7±1 ppb, which will be used as a reference for comparison in determining the increase in indoor ozone when the ES air cleaner is powered on.

Comparison of baseline indoor ozone levels in the UTT test houses

Figure 6 shows the baseline indoor levels of ozone in test house 1 and 2 for a period of 24 hours where the outdoor ozone levels were similar.

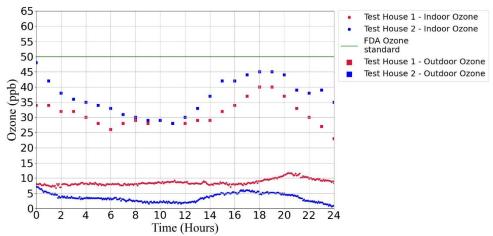


Figure 6. Ozone experiment 2 results for test houses #1 and #2.

Both houses are identical in layout, window to wall ratio, and size (factors related to infiltrations in a house envelope discussed in the *Infiltration of outdoor ozone into an indoors environment* section) with the only difference being that house #2 has a tighter envelope than house #1. Therefore, it can be expected that house #2 will have lower indoor ozone levels than house #1. As seen in the data from **Figure 6**, the indoor ozone levels in house #2 followed outdoor levels at a low concentration (around an average of 3.6±1 ppb), while the indoor levels in house #1 had a higher concentration (around an average of 8.6±1 ppb).

Figure 7 shows normalized results of both houses in continuity, where the vertical black line separates house #2 (hours 0-48) and house #1 (hours 48-168). The indoor and outdoor ozone levels are normalized in a range from zero to one in percentage of their highest value seen from house #1 and house #2.

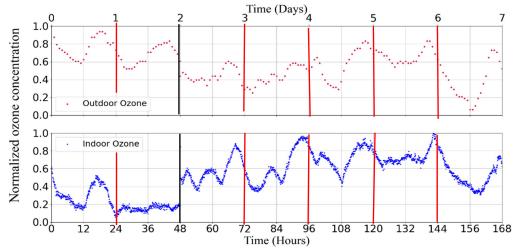


Figure 7. Normalized baseline indoor ozone levels for houses #1 (0-48 hours) and house #2 (48-168 hours).

Figure 7 provides a different visual for the same trends illustrated in Figure 6. House #2 is more air-tight, so the indoor ozone levels follow the outdoor ozone levels at a lower concentration than after the 48-hour mark when the ozone monitor was moved to test house #1.

Indoor ozone levels with mechanical ventilation in house #2

Figures 8 and 9 shows the indoor ozone data for ozone experiment 2, which had durations of 24 hours for ES air cleaners A and B respectively.

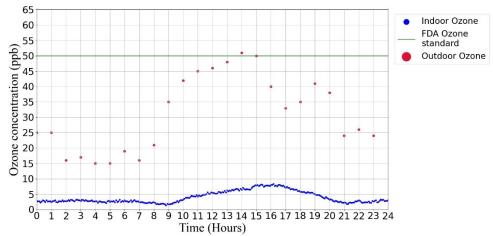


Figure 8. Ozone experiment 3 results during ES air cleaner A's testing.

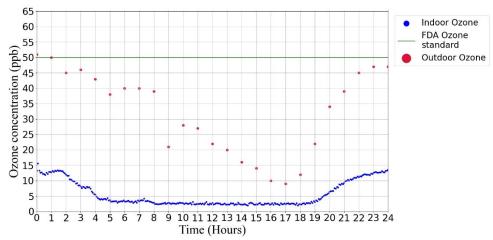


Figure 9. Ozone experiment 3 results during ES air cleaner B's testing.

As discussed in the *Infiltration of outdoor ozone into an indoors environment* section, infiltration through mechanical ventilation and natural ventilation (through the opening of windows and doors) lead to higher I/O ratios. Ozone experiment 3 observes indoor ozone levels in test house #2 based on the influence of mechanical ventilation. With the intake and exhaust ventilators in test house #2 being powered on, outdoor air is being directly introduced into the test house which has higher levels of ozone that is being mixed with indoor air. This causes the indoor ozone level trends to follow outdoor ozone level trends more closely. In terms of magnitude, the I/O ratio increases from baseline indoor ozone levels. One can see higher peaks (hour 16 in **Figure 8** and hours 0 and 24 in **Figure 9**) of indoor ozone levels in ozone experiment 3 as opposed to the peak in indoor ozone levels (hour 17 in **Figure 5**) seen in experiment 1.

Indoor ozone levels with mechanical ventilation and the powering on of two ES air cleaners in UTT test house #2

Figures 10 and 11 shows the indoor ozone data for ozone experiment 4, which had durations of 24 hours for ES air cleaners A and B respectively.

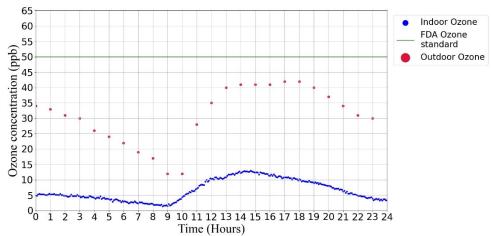


Figure 10. Ozone experiment 4 results during ES air cleaner A's testing.

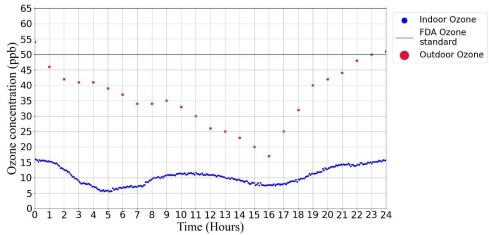


Figure 11. Ozone experiment 4 results during ES air cleaner B's testing.

In ozone experiment 4, outdoor ozone is still being directly introduced into the test house through mechanical ventilation. However, in this ozone experiment, there should be an indication that indoor ozone levels are higher than in ozone experiment 3 since the ES air cleaner is powered on. There is such an indication when comparing ozone experiments 3 and 4 for ES air cleaner A (**Figures 8 and 10**). Even though lower outdoor ozone levels are seen in ozone experiment 4 in comparison to experiment 3, the indoor ozone levels in the house were higher. A distinction also seen in the testing of ES air cleaner B (**Figures 9 and 11**) as there was a clear increase in indoor ozone from ozone experiment 3 to 4.

Indoor ozone levels with the powering on of two ES air cleaners in UTT test house #2

The results from ozone experiment 5 are shown in **Figures 12 and 13** for ES air cleaners A and B respectively. The duration of experiment 5 was 48 hours for ES air cleaner A and 72 for ES air cleaner B.

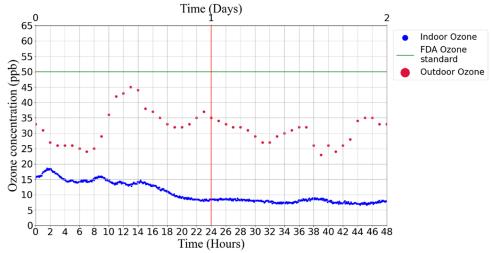


Figure 12. Ozone experiment 5 results during ES air cleaner A's testing.

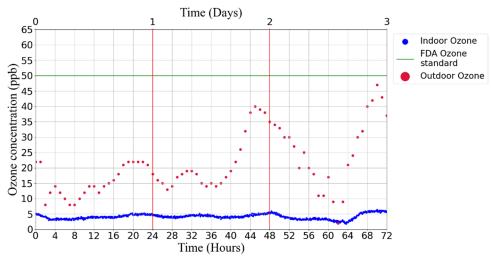


Figure 13. Ozone experiment 5 results during ES air cleaner B's testing.

Ozone experiment 5 allows for a clearer evaluation of the contribution that the ES air cleaners had in raising indoor ozone levels. In experiment 5, the only indoor sources of ozone are the ES air cleaners with indoor ozone levels being influenced by outdoor levels solely through infiltrations. This allows for a distinct comparison to made in indoor levels from ozone experiment 1 and experiment 5. For ES air cleaner A, the inside ozone levels vary around an average of 10.5±1 ppb (Figure 12) where in ozone experiment 1 they vary around an average of 2.7±1 pbb (Figure 5). ES air cleaner A, on max setting, has increased ambient ozone levels in the house by approximately 7±2 ppb. ES air cleaner B seems to increase the indoor ozone concentration only minimally from the ozone concentration in ozone experiment 1. ES air cleaner B contributed to indoor ozone levels that varied around an average of 4.2±1 ppb (Figure 13) leading to an approximate increase of approximately 1.5±2 ppb. As stated in Generation of ozone from ES air cleaners, ES air cleaners ionize incoming contaminant particles by generating a field of static electricity. In search of further reducing contaminant particles, different ES air cleaners will generate higher fields of static electricity based on respective design factors and/or higher operation options. Since ES air cleaner A has a maximum-on operation option while ES air cleaner B has an on option only, it is expected that ES air will generate more ozone due to a higher operation level of ionization. It is also important to note that the increases seen with ES air cleaner A and B were at the lower end of the 10-77 ppb increases mentioned in Generation of ozone from ES air cleaners.

Both air cleaners contributed to an increase in indoor ozone concentration. The statistical significance of the results was found with a hypothesis test for the difference of means. The averages of indoor ozone for experiment 5 (where the ES air cleaners were the only source of indoor zone) of both ES air cleaners were compared to average of indoor ozone in experiment 1 (baseline ozone levels). In order for the comparison of both ES air cleaners, data was taken from the first 48 hours of ES air cleaner A's Experiment 5 to match the sample size of ES air cleaner B's Experiment 5. The null hypothesis was that the ES air cleaners did not contribute to higher ozone levels than baseline ozone levels. Both ES air cleaners had p-values of less than 0.00001 when compared to baseline indoor ozone levels, which leads to the data results being statistically significant to a significance level of 95%. This leads to the conclusion that the average indoor ozone concentration is actually higher when the ES air cleaners are powered on and is not just a result due to chance. Nevertheless, the increase in indoor ozone from either of the ES air cleaners did not exceed the FDA 21CFR801 standard of 50 ppb mentioned in the *introduction*.

The results of the final ozone experiment, experiment 6, are shown in **Figures 14 and 15** for ES air cleaners A and B respectively. The duration of experiment 6 was 48 hours for ES air cleaner A and 72 for ES air cleaner B.

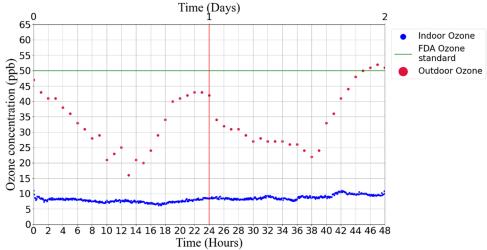


Figure 14. Ozone experiment 6 results during ES air cleaner A's testing.

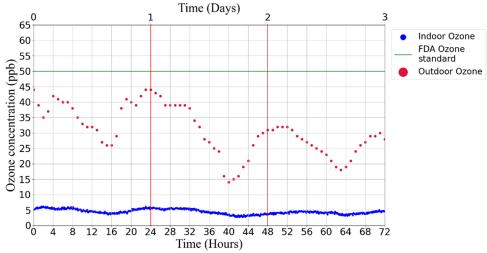


Figure 15. Ozone experiment 6 results during ES air cleaner B's testing.

Ozone experiment 6 focuses on whether there is a significant increase in ozone levels observed near the supply registers than when observed on the living space. The idea is that since the ES air cleaners are producing a by-product of ozone, ozone levels should be higher near the supply registers before diluting with the air in the living space. However, this did not seem to be the case. Indoor ozone levels for both ES air cleaners (**Figures 14 and 15**) seemed to remain very close to the indoor levels in ozone experiment 5 (**Figures 12 and 13**), when the sensor was on a stool. This concept is re-enforced in the stratification of ozone test discussed in *appendix B*.

Table 4 summarizes the results of the different ozone experiments.

Experiment	Result	
1	The average baseline concentration seen in test house #2 was 2.7 ppb.	
2	The average ozone levels in test house #2 (3.6 ppb) were 5 pbb lower than test house #1 (8.6 ppb).	
3	The average indoor ozone levels in test house #2 increased 1.3 and 2.9 ppb for ES air cleaners A and B, respectively from experiment 1 due to the powering on of mechanical ventilation.	
4	The average indoor ozone levels in test house #2 increased 2.6 and 5 ppb for ES air cleaners A and B, respectively from experiment 3 due to the powering on of the ES air cleaners and mechanical ventilation.	
5	The average indoor ozone levels in test house #2 increased 7.8 and 1.5 ppb for ES air cleaners A and B, respectively from experiment 1 just due to the powering on of the ES air cleaners.	
6	The average indoor ozone levels in test house #2 decreased 2.2 ppb and minimally increased by 1.5 ppb for ES air cleaners A and B, respectively from experiment 1 just due to the powering on of the ES air cleaners.	

Table 4. Summary of ozone experimental results.

CONCLUSIONS

The focus of this study was to evaluate the contribution that two ES air cleaners had in increasing indoor ozone concentrations in a research house with additional observations in different factors leading to higher indoor ozone levels. The experiments were conducted in two test houses, #1 and #2, at the University of Texas at Tyler. In test house #2, indoor ozone levels were compared when the ES air cleaners were powered on to baseline indoor ozone levels (indoor ozone levels with no indoor source of ozone and no ventilation). Both ES air cleaners contributed to increases in indoor ozone levels. ES air cleaner A showed an increase in ozone magnitude from 2.7 to 10.5 ppb (approx. 7±2 ppb), while ES air cleaner showed an increase from 2.7 ppb to 4.2 ppb (approx. 1.5±2 ppb). These results were statistically significant with very low p-values. Even though the ES air cleaners were found to give off a by-product of ozone, neither of the ES air cleaners surpassed the indoor ozone limit for FDA standard 21CFR801 with the ES air cleaner A and B being 39.5 and 45.8 ppb below the 50 ppb ozone limit, respectively. It is also noteworthy that the indoor ozone levels observed at the supply registers were not noticeably higher than ozone levels measured at the living space. The conclusions of the additional experimental findings are that, in test house #2, the powering on of ventilation lead to an increase in the indoor ozone levels from baseline levels with indoor ozone level trends following outdoor ozone level trends closely. An additional increase in indoor ozone levels were seen with the powering on of the ES air cleaners in tandem with mechanical ventilation then when just mechanical ventilation was powered on. Also, a comparison of baseline indoor ozone levels was made for test houses #1 and #2. Higher levels of indoor ozone were seen in test house #2 which has a tighter exterior envelope than test house #1.

NOMENCLATURE

Symbol and Acronyms	Description
UTT	University of Texas at Tyler
IAQ	Indoor Air Quality
ES	Electro-static
ppb	Parts-per-billion
HVAC	Heating, Ventilation, and Air Conditioning
PM	Particulate Matter
VOC	Volatile Organic Compounds
PCO	Photo-catalytic oxidizer
UV	Ultra-Violet
EPA	Environmental Protection Agency
FDA	Federal Drug Agency
BL	Boundary layer
I/O	Indoor/Outdoor
ACH	Air changes per hour
CARB	California Air Resources Board

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REFERENCES

- 1. Indoor Pollutants and Sources Basic Information on Pollutants and Sources of Indoor Air Pollution, EPA., https://nww.epa.gov/indoor-air-quality-iaq/introduction-indoor-air-quality#health (accessed Feb 2022)
- 2. Indoor Air Quality, BASF Catalysts, https://catalysts.basf.com/products-and-industries/indoor-air-quality (accessed Jan 2022)
- **3.** Bridger, C., The health impacts of indoor air quality, NEEF, https://www.neefusa.org/health/asthma/health-impacts-indoor-air-quality (accessed Feb 2022)
- 4. Collins, D. B., and Farmer, D. K. (2021) Unintended consequences of air cleaning chemistry, Environmental Science & Technology, 55, 18, 12172-12179. https://doi.org/10.1021/acs.est.1c02582
- 5. AQI Breakpoints, EPA, https://aqs.epa.gov/aqsweb/documents/codetables/aqi_breakpoints.html (accessed Dec 2022)
- 6. What is Ozone, EPA, https://www.epa.gov/ozone-pollution-and-your-patients-health/what-ozone (accessed Feb 2022)
- 7. Huang, Y., Yang, Z., and Gao, Z. (2019) Contributions of indoor and outdoor sources to ozone in residential buildings in Nanjing, *International journal of environmental research and public health*, 16(14), 2587. https://doi.org/10.3390/ijerph16142587
- **8.** CFR Code of Federal Regulations Title 21, FDA, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=801.415. (accessed Sep 2022)
- 9. Steenburgh, J., Lessons in boundary layer meteorology, Wasatch Weather Weenies, https://wasatchweatherweenies.blogspot.com/2017/10/lessons-in-boundary-layer-meteorology.html (accessed Feb 2022)
- 10. Jacob, J. D., Chilson, P. B., Houston, A. L., and Smith, S. W. (2018) Considerations for Atmospheric Measurements with Small Unmanned Aircraft Systems, atmosphere, 9(7), 252. https://doi.org/10.3390/atmos9070252
- 11. Petetin, H., Thouret, V., Athier, G., Blot, R., Boulanger, D., Cousin, J.-M., Gaudel, A., Nédélec, P., Cooper, O., Helmig, D., and Oltmans, S. J. (2016) Diurnal cycle of ozone throughout the troposphere over Frankfurt as measured by Mozaic-IAGOS commercial aircraft diurnal cycle of ozone throughout the troposphere, Elementa: Science of the Anthropocene, 4, 129. https://doi.org/10.12952/journal.elementa.000129
- 12. Lu, X., Zhang, and Shen, L. (2021) Chapter 2 Tropospheric ozone interacts with weather and climate, Air Pollution, Climate, and Health, 14-46. https://doi.org/10.1016/B978-0-12-820123-7.00006-1
- 13. Al-Jeelani, H. A. (2014) Diurnal and seasonal variations of surface ozone and its precursors in the atmosphere of Yanbu, Saudi Arabia, *Journal of Environmental Protection*, 5, 408-422. DOI: 10.4236/jep.2014.55044
- 14. Long-range transport of ground-level ozone and its precursors, cec.org, http://www.cec.org/files/documents/publications/2185-long-range-transport-ground-level-ozone-and-its-precursors-en.pdf (accessed Apr 2022)
- **15.** Koulougliotis, D., Kalimeris, A., Potozi, S., Lorilla, R.S., Kefala, G., and Nikolopoulos, D. (2015) Indoor Air Pollution: The Case of Ozone in Three Regions in Greece, *J Phys Chem Biophys*, 5, 1-5. DOI: 10.4172/2161-0398.1000191
- 16. Ground-level Ozone Basics, EPA, https://www.epa.gov/ground-level-ozone-pollution/ground-level-ozone-basics (accessed Apr 2022)
- 17. Gorai, A. K., Tuluri, F., Tchounwou, P. B., and Ambinakudige, S., (2015) Influence of Local Meteorology and NO2 Conditions on Ground-Level Ozone Concentrations in the Eastern Part of Texas, USA, *Air Quality, Atmosphere & Health*, 8, 81–96. DOI: 10.1007/s11869-014-0276-5
- **18.** Lai, D., Karava, P., and Chen, Q. (2015) Study of Outdoor Ozone Penetration into Buildings through Ventilation and Infiltration, Building and Environment, 93 (2), 112-118. https://doi.org/10.1016/j.buildenv.2015.06.015.
- **19.** Ma, N., Hakkarainen, M., Hou, M., Aviv, D., and Braham, W.W. (2021) Impacts of building envelope design on indoor ozone exposures and health risks in residential houses. http://dx.doi.org/10.2139/ssrn.3972626
- 20. Okubo, M., Yamamoto, T., Kuroki, T., and Fukumoto, H., (2001) Electric Air Cleaner Composed of Nonthermal Plasma Reactor and Electrostatic Precipitator, *IEEE Transactions in Industry Applications*, 37(5), 1505-1511. DOI: 10.1109/28.952528
- 21. Lu, Z., Sun, W., Li, C., Cao, W., Jing, Z., Li, S., Ao, X., Chen, C., and Liu, S. (2020) Effect of granular activated carbon pore-size distribution on biological activated carbon filter performance, Water Research, 177, 115768, https://doi.org/10.1016/j.watres.2020.115768.
- 22. Boelter, K. J., and Davidson, J. H., (1997) Ozone generation by indoor, Electrostatic Air Cleaners, *Aerosol Science and Technology*, 27(6), 689-708, https://doi.org/10.1080/02786829708965505
- 23. Afshari, A., Ekberg, L., Forejt, L., Mo, J., Rahimi, S., Siegal, J., Chen, W., Wargocki, P., Zurami, S., and Zhang, J., (2020) Electrostatic Precipitators as an Indoor Air Cleaner—A Literature Review, Sustainability, 12, 8774; doi:10.3390/su12218774
- 24. Residential air cleaners: A technical summary, EPA, https://www.epa.gov/indoor-air-quality-iaq/guide-air-cleaners-home (accessed Dec 2022)
- **25.** Poppendieck, D. G., Rim, D., and Persily, A. K., (2014) Ultrafine Particle Removal and Ozone Generation by In-Duct Electrostatic Precipitators, *Environ. Sci. Technol*, 48, 2067–2074, *dx.doi.org/10.1021/es404884p*

26. Allen, R. J., Wadden, R. A., and Ross, E. D. (1978) Characterization of potential indoor sources of ozone, *American Industrial Hygiene Association Journal*, 39(6), 466-471, https://doi.org/10.1080/0002889778507791

ABOUT STUDENT AUTHOR

Giovanni Cerrato is a recent graduate from the University of Texas (UT) at Tyler. He graduated with a bachelor's degree in mechanical engineering and will seek his master's degree in mechanical engineering at UT Tyler in the academic school year of 2022-2023. He plans to continue to do research in the space of indoor air quality and will seek to work in the HVAC industry.

PRESS SUMMARY

This study reveals the contributions of two electrostatic air cleaners (installed in an HVAC system) in raising ozone levels in a test home by releasing ozone as a by-product. Both electrostatic air cleaners contributed to raising ozone levels, however neither raised the ozone levels above the indoor limit of ozone set by FDA standard 21CFR801.

APPENDIX A - OZONE MONITOR ZERO CHECK

To ensure the quality of the ozone monitor data, the ozone monitor was periodically zeroed out throughout the experiments. The zero check was performed by zeroing the ozone monitor around a reference point of zero ozone with the use of an ozone scrubber provided by the manufacturer (see **Figure 1A**). The ozone scrubber was attached to the ozone monitor reading input (see **Figure 2A**). The ozone monitor was then calibrated to read 0 ppb with the ozone scrubber on. The ozone scrubber was then removed to make readings, and the calibration is complete.



Figure 1A. Ozone scrubber.



Figure 2A. Ozone scrubber attached to ozone monitor.

APPENDIX B - JUSTIFICATION FOR THE HORIZONTAL AND VERTICAL PLACEMENT OF THE OZONE MONITOR

The location of the ozone monitor was determined as the result of a stratification test and a test that measured the critical point of ozone in the test house. The ozone monitor was placed on a stool at the center of three bedrooms and set to read indoor ozone levels. The results are shown in **Table 1B**.

Room	Ozone (ppb)
Master Bedroom	2.8
Bedroom 2	2.9
Bedroom 3	2.7

Table 1B: Critical point of ozone test

The room location of the ozone monitor seemed to minimally affect the ozone readings. For this reason, the sensor was placed in a centralized location in the house in the living room.

The stratification test measured ozone concentrations at different heights from the ground. The ozone monitor was raised in increments of 11.25 inches by being placed on ladder steps. The results are shown in **Table 2B**.

Height (Inches)	Ozone (ppb)
11.25	2.8
22.50	3.0
33.75	3.5
45.00	3.6
56.25	3.6
67.50	3.4

Table 2B: Stratification of ozone tes

The critical point of ozone was around 45-56 inches from the ground. This is considered "waistline" level. For this reason, the ozone monitor was placed on a stool in that height range.

Student Perceptions of Instructor-Student Rapport and Motivation In Hybrid Courses During COVID-19

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ABSTRACT

The relationship between instructors and their students is essential for developing a classroom climate where students feel motivated to learn. The current study surveyed 658 undergraduate students to examine the relationship between instructor-student rapport and motivation in online and face-to-face classes during the COVID-19 pandemic. Results indicated (1) students experienced more rapport with their instructors during face-to-face classes compared to their online classes, (2) students perceived their motivation was greater during face-to-face classes than in online classes, and (3) there was a significant positive relationship between instructor-student rapport and student motivation in both online and face-to-face classes. This study's findings lend further support to research that emphasizes the importance of creating a sense of community in online classes, where students feel connected to their instructors and, consequently, motivated to learn.

KEYWORDS

Instructor-Student Rapport; Motivation; Hybrid Courses; COVID-19; Online Learning

INTRODUCTION

The primary goal of instructors is to encourage their students to learn.¹ Researchers have found that positive instructor-student relationships play an essential role in creating learning environments where students feel connected, involved, and motivated during class.¹² Developing rapport with students helps instructors meet the goal of fostering an effective learning environment. However, developing this type of relationship with students can be challenging for instructors when there is a drastic change in their conventional teaching methods.

Amidst the COVID-19 pandemic, many universities adopted online learning as the primary form of instruction to comply with the pandemic's safety protocols. Due to this shift, the number of students enrolled in remote courses drastically increased. In addition, the trend to participate in distance learning has been increasing over the past decade. The US National Center for Education Statistics (2018) reported approximately seven million postsecondary students in the United States were enrolled in online courses. As we move forward into a more technology-driven educational climate, it is crucial to understand how the change from traditional face-to-face instruction to different forms of online instruction can impact students' learning environment. Therefore, the purpose of this study was to assess the differences in students' perceptions related to instructor-student rapport and student motivation during students' online classes versus face-to-face classes.

Online learning

Research has struggled to find an agreed-upon definition of online learning.³ Considering the online learning literature, Singh and Thurman³ developed the following definition: "Online education is defined as education being delivered in an online environment through the use of the internet for teaching and learning. This includes online learning on the part of the students that is not dependent on their physical or virtual co-location. The teaching content is delivered online, and the instructors develop teaching modules that enhance learning and interactivity in the synchronous or asynchronous environment" (p. 302).

In theory, online learning can be just as effective as traditional face-to-face instruction when appropriately implemented. ⁴ Online learning has many benefits for both instructors and students. In times of crisis, such as the COVID-19 pandemic, it provides a student-centered learning environment that is flexible in time and location. ⁵ It is an innovative instructional method that allows instructors to adapt to different learning styles that are more inclusive, which differ from the traditional lecture format used during face-to-face classes. According to Dhawan, ⁵ online learning provides teachers with tools to build a collaborative and interactive learning environment.

COVID-19 and Higher Education

On March 12, 2020, the World Health Organization declared the COVID-19 pandemic. The virus spread rapidly across the globe and has affected the livelihoods of billions of people. In the United States, academic institutions had to comply with social distancing guidelines and a mandatory quarantine imposed by the government to keep citizens safe. In the state of emergency and uncertainty, academic institutions had to find a way to continue education for college students whilst complying with these guidelines. As a result of the pandemic, most academic institutions across the nation had to switch to online learning for the first time. The virus spread rapidly across the globe and has affected the livelihoods of billions of people. In the United States, academic institutions had to comply with social distancing guidelines and a mandatory quarantine imposed by the government to keep citizens safe. In the state of emergency and uncertainty, academic institutions had to find a way to continue education for college students whilst complying with these guidelines. As a result of the pandemic, most academic institutions across the nation had to switch to online learning for the first time.

Literature regarding the transition to online instruction during COVID-19 has found that instructor readiness and willingness to change to an online course format is a significant determinant for their success.⁸ According to Veletsianos and Seaman,⁷ amidst COVID-19, many instructors had to tackle the challenge of teaching online without previous experience and with very little time to prepare. Similarly, college students who were accustomed to traditional face-to-face classes had to adapt to online classes quickly.

Aboagye et al. 9 found that during the transition to online classes, students' most serious obstacles was the lack of access to the technology required for their courses, such as equipment and Internet access. Moreover, the most significant challenge impacting students' intentions to study online was lecturer issues. 9 Among the many issues that students faced, the one that impacted their experience the most was the quality of instruction they were receiving during the pandemic. Nonetheless, the emergency switch to online classes was a first-hand experience and challenge for the education system as a whole, from the academic institutions themselves to the faculty and students.

Motivation

According to cognitive psychology research, motivation is a part of the learning process.⁵ Motivation increases attention and "sets the stage for cognitive engagement" (p. 476). Students who feel motivated during a class may be more likely to succeed. For many years, scholars defined motivation as trait-oriented, meaning that students' self-efficacy, goals, and interests determined their motivation. However, research suggests that external factors such as course format and instructor immediacy behaviors influence student motivation as well. Christophel described this type of motivation as *state-oriented*.

The self-determination theory of motivation, developed by Deci and Ryan,¹³ suggests it is important to consider the different factors that influence an individual's psychological needs to understand human motivation. Deci and Ryan¹³ defined these needs as "innate psychological nutrients that are essential for ongoing psychological growth, integrity, and well-being" (p. 229). Moreover, these needs are classified by intrinsic and extrinsic factors. Intrinsic motivation, similar to trait-oriented motivation,² is self-determined or autonomous behavior. In other words, when individuals act upon intrinsically motivated behavior, they do not need reinforcement. Behaviors led by intrinsic factors are in themselves rewarding for an individual.^{13,15}

In contrast to the self-determination theory of motivation, extrinsic motivation refers to behaviors that are influenced by specific external factors. This type of motivation is also known as controlled motivation. It depends upon circumstances such as instructor approval, external validation, shame avoidance, fear of consequences, classroom environment, and more. In other words, extrinsic motivation is behavior dependent on circumstances regulated by other people or conditions. ^{13,14,15}

The current study examined student motivation among participants who were taking hybrid courses, which are designed to alternate between online and face-to-face instruction. The COVID-19 policies at the university where this study took place allowed faculty to hold face-to-face, online, and/or hybrid classes, so students were exposed to an array of experiences. When motivation is mentioned in this study, it refers to extrinsic motivation because the researchers focused on measuring motivation in the context of two types of classroom conditions. Lastly, motivation is necessary to examine in educational environments because it leads to an instructor's ultimate goal – learning.² Another important factor for the learning process, in addition to motivation, is instructor-student rapport, which is discussed in the following section.

Instructor-Student Rapport

Frisby and Housley Gaffney¹⁶ defined instructor-student rapport as the overall perception that students have of their instructors and "the belief that there is a mutual, trusting, and prosocial bond, including personal connection and enjoyable interactions" (p. 341). Instructors that build a healthy rapport with their students are seen as developing an effective learning environment.^{1,16} It is important to understand if and how much students care about their relationships with instructors because it can provide insight into students' relational goals in the classroom.

Although rapport and immediacy are two different constructs¹⁶ rapport has been used as an umbrella term that includes instructor immediacy behaviors¹⁷ Immediacy behaviors are verbal or nonverbal communication strategies that create affinity between instructors and students.¹⁸ Instructor-student rapport researchers have focused on examining the relationship between instructor

immediacy behaviors and student motivation.^{2,19,20} These scholars have found that a positive relationship exists between instructor-student rapport and student motivation. Moreover, other scholars have suggested that instructor-student rapport, including teacher immediacy behaviors, influence student motivation to learn important lesson content.^{2,17,18} Ultimately, we are uncertain how this relationship could vary in the context of online and face-to-face classes.

Virtual Hybrid Learning

The university examined in this study implemented Virtual Hybrid Learning (VHL) as the primary instruction mode during the COVID-19 pandemic. Virtual Hybrid Learning is a mixed-mode of instruction that combines online learning with traditional face-to-face instruction synchronously.^{22,29} Zydney et al.²³ described this mode of instruction as the "Here or There" (HOT) approach, meaning some students partake in class on-campus (i.e., Here) and other students join in a remote location (i.e., There) at the same time. It is important to consider the instruction modes experienced by students in this study because they are a different experience from courses that are solely online or face-to-face. The educational environment in pure online learning and VHL is not the same. With VHL, students have the opportunity to interact with their instructors in person on the days they meet for face-to-face instruction.

Hybrid Learning

Another mode of instruction implemented at the university in this study was hybrid learning, also known as "blended learning." Similar to VHL, this mode combines online with face-to-face instruction. ²⁴ In hybrid learning, students meet with instructors interchangeably, either solely online or in person. Professors using hybrid learning choose the days they want to meet with students in-person or online. With VHL and hybrid learning, participants in this study have experienced online and in-person classes. Some studies have found no significant differences in the academic outcome and the learning environment between online and face-to-face classes. ^{25,26} However, Lyke and Frank²⁷ found students feel less satisfied with their educational experience in online classes. Still, the learning environment in full online and face-to-face classes cannot be compared to the online and face-to-face learning environment in VHL or hybrid learning courses.

A limited amount of empirical research has examined the educational environment differences between online and face-to-face classes for students in VHL or hybrid learning course formats. ^{28,29} One study on VHL found that online students experience significantly low levels of "relatedness" compared to face-to-face students. ²¹ Given the literature gap regarding the educational environment in VHL and hybrid courses, there is uncertainty about the differences in how students experience their online and face-to-face classes within VHL/hybrid learning format. Specifically in terms of their rapport with instructors and motivation.

Overall, previous literature on VHL and hybrid learning formats suggests a lack of student connectedness and rapport associated with classes that take place online. ^{21,28,29} Moreover, previous research has found that instructor-student rapport is positively related to motivation. ^{2,12,19,20} Based on the literature, it is expected that students will report a lack of rapport with instructors in their online classes and report less motivation in their online classes compared to their face-to-face classes. Thus, we posit the following hypothesis:

 On average, students will report higher rapport and motivation ratings in their face-to-face classes compared to their online classes.

Taking into account the literature discussed, we formulated five research questions aimed at understanding students' experiences with hybrid courses during COVID-19:

- 1. To what degree do students value their relationships with instructors?
- 2. Is instructor-student rapport in online classes positively related to student motivation in online classes?
- 3. Is instructor-student rapport in face-to-face classes positively related to student motivation in face-to-face classes?
- 4. Is student motivation in online classes negatively related to motivation in face-to-face classes?
- Is instructor-student rapport in online classes negatively related to instructor-student rapport in face-to-face classes?

METHODS AND PROCEDURES

Participants

Participants in this study consisted of 658 undergraduate students attending a private, Southern university who were taking hybrid classes (face-to-face and online) and/or virtual hybrid classes, depending on their professors' preferences. The participants consisted of 574 women, 76 men, six non-binary/third gender students, and two who selected "other." Students' age ranged from 18 years of age to 61 years of age (M = 20.13, SD = 3.06). The participants were of various class standings, including 194 first-year students, 167 sophomores, 140 juniors, 156 seniors, and one student who did not report a class standing. There were 476 students who identified as Non-Hispanic White, 22 who identified as Non-Hispanic Black, 106 who identified as Hispanic White, nine who identified as Hispanic Black, one who identified as Native American or American Indian, 22 who identified as Asian/Pacific Islander, and three who identified as Middle Eastern. Nineteen students did not report cultural backgrounds.

Procedures

Data were collected using an IRB-approved Qualtrics survey distributed via email through a global university message to undergraduate students enrolled in Spring 2021 courses. Students were incentivized to participate in the survey with a \$25 gift card raffle. The Institutional Review Board at our university granted this study "exempt status" as described in 45 CFR 46.104 of the Department of Health and Human Services Policy for the Protection of Human Subjects.

Previous scale construction research on motivation and rapport in the classroom 1,2,17 was used to create the current study's survey and scales. The scales in the survey focused on measuring the following concepts: (a) student value of instructor rapport, (b) instructor-student rapport in online classes, (c) instructor-student rapport in face-to-face classes, (d) student motivation in online classes, and (e) student motivation in face-to-face classes. Cronbach's alpha is the reliability coefficient that was used to measure the internal consistency and reliability of the survey items in each scale. Each item in the survey was presented on a Likert-type scale where participants were given the opportunity to choose an answer that reflects their degree of agreeableness with each item on the survey.

MEASURES

Student Value of Instructor Rapport

Knowing the extent to which students care about their instructors is key to understanding relational goals in the classroom. This scale was created to measure how much students care about their instructors. This scale consisted of a five-item on a five-point Likert-type scale. The scale ranged from "strongly agree" (5) to "strongly disagree" (1). The Cronbach alpha for this scale was .83 (M = 3.76, SD = .77). See **Table 1** below for the scale items.

Iter	ns	
	1.	I value my relationships with professors.
2.		Having close relationships with my professors is important to me.
3.		I have one or more professors whom I see as a mentor, and this is important to me.
4.		When I have good or bad news to share, I often share it with my professors.
5.		I strongly care about my professors.

Table 1. Value of instructor rapport.

Instructor-Student Rapport Online

To understand students' current perceptions of rapport with instructors during their online classes, a five-item on a five-point Likert-type scale that ranged from "strongly agree" (5) to "strongly disagree" (1) was created. The Cronbach alpha for this scale was $.76 \ (M = 3.15, SD = .87)$. See **Table 2** below for the scale items.

Iter	Items				
	1.	I have a close relationship with my professors this semester.			
2.		I look forward to seeing my professors via Zoom.			
3.		I am comfortable interacting with my professors during online classes.			
4.		I feel more connected to professors taking online classes than when taking in-person classes.			
5.		I consider my professors approachable this semester.			

Table 2. Instructor-student rapport online.

Motivation in Online Classes

The current study assessed students' motivation during their online classes. The scale consisted of seven items on a five-point Likert-type scale that ranged from "strongly agree" (5) to "strongly disagree" (1). The Cronbach alpha for this scale was .86 (M = 2.72, SD = .95). See **Table 3** below for the scale items.

Items					
1.	Overall, I feel motivated to attend my Zoom lectures.				
2.	I find my classes that take place through zoom interesting.				
3.	I often feel enthusiastic about attending my online classes.				
4.	I feel excited during online classes.				
5.	I am involved during my online classes.				
6.	Online classes are challenging.*				
7.	I am more likely to skip classes that take place online.*				

Table 3. Motivation in online classes.

Instructor-Student Rapport Face-to-Face.

Participants reported their perceptions of rapport with instructors during face-to-face classes. This scale consisted of a four-item five-point Likert-type scale that ranged from "strongly agree" (5) to "strongly disagree" (1). The Cronbach alpha for this scale was .86 (M = 4.20, SD = .84). See **Table 4** below for the scale items.

I had close relationships with my professors in semesters where courses were taught fully in person.
I look forward to seeing my professors in-person.
I feel comfortable interacting with my professors during classes that take place in person.
I feel more connected to professors taking in-person classes than when taking online classes.

Table 4. Instructor-student rapport face-to-face.

Motivation Face-to-Face.

The purpose of this scale was to assess student motivation during their current in-person classes or in past semesters where classes took place fully in-person. This scale consisted of seven items on a five-point Likert-type scale that ranged from "strongly agree" (5) to "strongly disagree" (1). The Cronbach alpha for this scale was $.92 \ (M = 3.97, SD = .8)$. See **Table 5** below for the scale items.

Items	
1.	I feel motivated to attend my in-person classes.
2.	I often feel enthusiastic about attending classes that take place face-to-face.
3.	I feel excited during my in-person classes.
4.	I am involved during my in-person classes.
5.	I participate more during in-person classes than online classes.
	Table 5 Motivation in face-to-face classes

 Table 5. Motivation in face-to-face classes

Data Analysis

In order to compare the average difference between the variables of interest in research question one and the hypothesis, the mean difference was used to examine how the variables differed from one another. Doing this allowed the authors to reflect on the average level of agreeableness reported by students for each Likert-type scale. Moreover, SPSS software was used to determine Pearson product-moment correlations to examine the relationships between the variables of interest in the remaining research questions (3 - 5).

RESULTS

The first research question was asked to understand how much students care about their relationships with professors. The mean score for the value of the instructor rapport scale was 3.76 (SD = .77). This mean score reported students, on average, "somewhat agree" that they care about their relationships with instructors.

Research question two asked if instructor-student rapport in online classes positively correlates with student motivation in online classes. A Pearson product-moment correlation found a significant and positive association between instructor-student rapport and motivation in online classes (r = .635, p < .01). Similarly, research question three asked if instructor-student rapport in face-to-face classes positively correlates with student motivation in face-to-face classes. A Pearson product-moment correlation found a significant and positive association between instructor-student rapport and motivation in face-to-face classes (r = .809, p < .01). See **Table 6** for correlations.

Research question four asked if there is a negative relationship between student motivation in online and face-to-face classes. A Pearson product-moment correlation found a significant and negative association between student motivation in online and face-to-face classes (r = -.351, p < .01). Therefore, students who feel motivated in one-course format feel less motivated in the latter. See **Table 6** for correlations.

Furthermore, research question five asked if there is a negative association between instructor-student rapport in online versus face-to-face classes. This Pearson product-moment correlation revealed no significant relationship between these two variables (r = .-063, p < .107). In other words, students generally have a degree of rapport with their instructors in both online and face-to-face classes.

		M	SD	а	1	2	3	4
1.	Value of Instructor Rapport	3.76	.77	.83				
2.	Instructor-Student Rapport Online	3.15	.87	.76	.375**			
3.	Motivation Online	2.72	.95	.87	.119**	.635**		
4.	Instructor-student Rapport Face-to-Face	4.20	.84	.86	.495**	063	331**	
5.	Motivation Face-to-Face	3.97	.88	.92	.413**	072	351**	.809**

^{**} Significant at the p < 0.01 level.

Table 6. Correlations, means, and standard deviations of variables.

The hypothesis posited that, on average, students would report significantly less motivation and rapport with instructors during their online classes than in their face-to-face classes. On the Instructor-Student Rapport Online scale, most students reported no negative or positive rapport with instructors (M = 3.15, SD = .87). In contrast, relating to the instructor-student rapport face-to-face scale, students reported they feel a strong rapport with their instructors during face-to-face classes (M = 4.20, SD = .84). There is a considerable difference between these two scales. The mean difference (MD = 1.05) suggests students experience higher rapport with instructors during their face-to-face classes than in their online classes. Moreover, students reported feeling neutral in their motivation during online classes (M = 2.72, SD = .95). In contrast, in the Motivation Face-to-Face scale, students reported "somewhat agree" they felt motivated in their face-to-face classes (M = 3.97, SD = .88). The mean difference for these scales (MD = 1.25) suggests that students tend to be significantly more motivated during their face-to-face classes than in their online classes. Thus, the hypothesis was supported.

DISCUSSION

This study sought to examine the differences in instructor-student rapport and motivation during online and face-to-face classes. This study's overall goal was to survey students' perceptions of their online learning experience during the COVID-19 pandemic. When students are taking online classes, they do not perceive the same rapport with instructors in comparison to their in-person classes. In addition, the learning environment during online classes tends to be less motivating compared to face-to-face classes. This study contributes to educational technology advances by offering another piece of research that addresses some shortcomings with online classes in VHL and hybrid learning course formats.

When instruction is shifted to an online format, it becomes an entirely different educational environment because technology is used as a medium for communication.³⁰ Instructors are encouraged to approach online classes differently from face-to-face classes.⁵ The students in this study reported significantly lower levels of rapport with instructors during online classes. These

findings support Butz & Stupnisky's²⁹ study, where they found that online students experience less "relatedness" with instructors than face-to-face students in VHL courses. Crim³¹ suggested this phenomenon can occur because it is often easy for students in online classes to accept and observe course material passively instead of engaging with their instructors and other students in class.

There is a likelihood that students perceive a lack of social presence with online classes due to the change of medium, which involves computer-mediated- communication through conference software. Tu and McIsaac³² described social presence as "the degree of awareness of another person in an interaction and the consequent appreciation of an interpersonal relationship" (p. 133). Therefore, the degree of social presence experienced in online learning can differ significantly from that in face-to-face classes, influencing students' perceptions of instructor rapport.

Similar to early findings by Christophel,² this study's findings suggest that as student rapport with instructors increases, so does their motivation in class. As a result, the findings from our study support previous literature that suggests a positive association between instructor-student rapport and student motivation. ^{2,12,19,20} This positive relationship can exist because when students feel that their instructors care about them and their educational goals, it helps students develop greater self-determination. ¹⁴

Findings from this study suggest students who feel motivated in face-to-face classes are significantly less likely to feel motivated in online classes. Fritz et al.³³ also found a relationship between student motivation and their learning style. Learning style is a concept that posits individuals process and retains information differently.³⁴ The researchers suggest that students who are in a class format that does not fit their learning style are likely to feel unmotivated.³³ Similarly, students in this study who reported feeling more motivated in a particular class format could have simply had a preference towards it. There are significant differences in the class format for face-to-face and online classes, which can explain the significant negative relationship found between motivation in online versus face-to-face classes.

According to Murdock and Williams²⁵, there are no significant differences in learning outcomes for students that take online and in-person classes. However, there is a gap in the literature regarding student experiences with different learning environments, such as VHL/hybrid classes.^{28,29} Addressing this gap, this research found significant differences in the levels of rapport and motivation during face-to-face and online classes.

It is crucial to consider that this study took place within hybrid/VHL classes that were implemented as an emergency protocol to continue education for students amidst the COVID-19 pandemic. Instructors and students were undergoing a distinctive circumstance that influenced their experience in the classroom. In retrospect, taking VHL classes voluntarily, and taking them as an emergency adaptation to continue education are two separate conditions and should be studied as such.³⁵

Instructor immediacy behaviors within the context of a pandemic could be distinct in that they can be influenced by rare interactions that are particular to the emergency. In other words, instructor-student rapport can be determined, for example, by an instructor's empathy when a student is sick or when a student is undergoing mental health problems due to the cathartic effects of being quarantined. Furthermore, rapport could be influenced by an instructor's leniency with assignments or their understanding for students who are having technological difficulties. Ultimately, instructor-student immediacy behaviors during national emergencies should be further explored and examined.

Limitations

This research was not without its limitations. First, female participants were disproportionately represented in the sample gathered for this study. The link to this survey was distributed in a message sent to all undergraduate students using the university's "global email." Future research should aim to gather a sample that more accurately represents the gender/sex ratio of the university where the study is conducted.

Students have experienced significant shifts in their education amidst the COVID-19 pandemic. Therefore, this study has a limitation that considers the mediating role of the psychological distress on students caused by the pandemic. 40 Thus, the survey responses could have been negatively biased towards online classes due to the cathartic effects of the current educational climate. Future research should try to find ways to avoid any biases that could impact students' responses.

The last limitation is that the survey did not assess precisely how many face-to-face or online classes students took during the semester. Therefore, we are not certain of how many hybrid or VHL courses students were taking and how this may have affected their responses to the survey. Furthermore, this study did not access students' majors and their interests. As this study focused on measuring student state-oriented motivation, the nature of different course subjects could also influence student motivation. In future studies, researchers should assess students in more depth about their enrolled courses.

Implications

This study's results lend further support to scholars who have emphasized the importance of fostering a sense of community among online students. ^{31,36,37} Research suggests that instructors can build a strong social presence with students through collaboration, meaningful interactions, and participation during online classes. ^{25,38,39} Moreover, instructors should adapt their conventional face-to-face class format to fit an effective student-centered online learning environment. ⁵ These are all factors that help students build rapport with instructors and their peers during online classes. Therefore, given the findings, we suggest instructors prioritize developing a strong rapport with their students because it can enhance their motivation to learn, whether it be face-to-face or online. Moreover, institutions should consider the findings from this study when training their faculty on adjusting their curriculum and teaching style during emergencies or public health crises. Future research should aim to examine important instructor-student immediacy behaviors in the context of an emergency switch to online learning. This is important because it will allow institutions and instructors to continue offering the same quality of education regardless of external circumstances. It will also allow students to receive the same level education no matter where they are or the circumstances they are enduring.

CONCLUSIONS

The present study examined instructor-student rapport and student motivation during online and face-to-face classes in VHL/hybrid learning courses. This study was conducted during the COVID-19 pandemic, reflecting on the current educational climate in higher education. Taken together, the findings support a positive relationship between instructor-student rapport and student motivation. Student motivation and instructor-student rapport are seen as greater in face-to-face classes than in online classes. Further research should continue to examine the educational environment in virtual hybrid learning courses and hybrid courses so students may experience consistency in their educational experience.

REFERENCES

- 1. Frisby, B. N., & Martin, M. M. (2010) Instructor–student and student–student rapport in the classroom. *Communication Education*, 59(2), 146-164.
- 2. Christophel, D. M. (1990) The relationships among teacher immediacy behaviors, student motivation, and learning. *Communication education*, 39(4), 323-340.
- 3. Singh, V., & Thurman, A. (2019) How many ways can we define online learning? A systematic literature review of definitions of online learning (1988-2018) *American Journal of Distance Education*, 33(4), 289-306.
- 4. Anderson, T. (Ed.) (2008) The theory and practice of online learning. Athabasca University Press.
- 5. Dhawan, S. (2020) Online learning: A panacea in the time of COVID-19 crisis. *Journal of Educational Technology Systems*, 49(1), 5-22.
- **6.** World Health Organization. (2020). *Coronavirus disease 2019 (COVID-19)*: situation report, 50. *World Health Organization*. https://apps.who.int/iris/handle/10665/331450
- 7. Johnson, N., Veletsianos, G., & Seaman, J. (2020) US Faculty and Administrators' Experiences and Approaches in the Early Weeks of the COVID-19 Pandemic. Online Learning, 24(2), 6-21.
- Ali, W. (2020) Online and remote learning in higher education institutes: A necessity in light of COVID-19 pandemic. Higher education studies, 10(3), 16-25.
- 9. Aboagye, E., Yawson, J. A., & Appiah, K. N. (2021) COVID-19 and E-learning: The challenges of students in tertiary institutions. *Social Education Research*, 1-8.
- 10. Blumenfeld, P. C., Kempler, T. M., & Krajcik, J. S. (2006) Motivation and cognitive engagement in learning environments. na.
- 11. Wigfield, A., Turci, F.L., Cambria, J., Eccles, J. S. (2012) Motivation in education. *The Oxford handbook of human motivation*, 443-461.
- **12.** King, R. B., & McInerney, D. M. (2016) Culturalizing motivation research in educational psychology. *British Journal of Educational Psychology*, 1-7.
- **13.** Deci, E. L., & Ryan, R. M. (2000) The" what" and" why" of goal pursuits: Human needs and the self-determination of behavior. Psychological inquiry, 11(4), 227-268.
- 14. Deci, E. L., & Ryan, R. M. (2008) Self-Determination Theory: A Macrotheory of Human Motivation, Development, and Health. *Canadian Psychology*, 49(3), 182-185.
- **15.** Ryan, R. M., & Deci, E. L. (2020) Intrinsic and extrinsic motivation from a self-determination theory perspective: Definitions, theory, practices, and future directions. *Contemporary Educational Psychology*, 61, 101860.
- **16.** Frisby, B. N., & Housley Gaffney, A. L. (2015) Understanding the role of instructor rapport in the college classroom. *Communication Research Reports*, *32*(4), 340-346.
- 17. Wilson, J. H., Ryan, R. G., & Pugh, J. L. (2010) Professor-student rapport scale predicts student outcomes. *Teaching of Psychology*, 37, 246–251.
- **18.** Baringer, D. K., & McCroskey, J. C. (2000) Immediacy in the classroom: Student immediacy. *Communication education*, 49(2), 178-186.

- **19.** Estepp, C. M., & Roberts, T. G. (2015) Teacher Immediacy and Professor/Student Rapport as Predictors of Motivation and Engagement. *NACTA Journal*, *59*(1).
- 20. Komarraju, M., Musulkin, S., & Bhattacharya, G. (2010) Role of student–faculty interactions in developing college students' academic self-concept, motivation, and achievement. *Journal of college student development*, 51(3), 332-342.
- 21. Butz, N. T., Stupnisky, R. H., Peterson, E. S., & Majerus, M. M. (2014) Motivation in synchronous hybrid graduate business programs: A self-determination approach to contrasting online and on-campus students. *Journal of Online Learning & Teaching*, 10(2), 211-227.
- 22. Hastie, M., Hung, I. C., Chen, N. S., & Kinshuk, (2010) A blended synchronous learning model for educational international collaboration. *Innovations in Education and Teaching International*, 47(1), 9–24.
- 23. Zydney, J. M., McKimmy, P., Lindberg, R., & Schmidt, M. (2019) Here or there instruction: Lessons learned in implementing innovative approaches to blended synchronous learning. *TechTrends*, 63(2), 123-132.
- 24. Means, B., Toyama, Y., Murphy, R., & Baki, M. (2013) The effectiveness of online and blended learning: A meta-analysis of the empirical literature. *Teachers College Record*, 115(3), 1-47.
- **25.** Williams, A. M. & Murdock, J. L. (2011) Creating an online learning community: Is it possible? *Innovative Higher Education*, *36*(5), 305-315.
- 26. Russel, T. (1999) "The No Significant Difference Phenomenon." Web site. Teleeducation. nb. ca/nosignificantdifference/.
- 27. Lyke, J., & Frank, M. (2012) Comparison of student learning outcomes in online and traditional classroom environments in a psychology course. *Journal of Instructional Psychology*, 39(3-4), 245-251.
- 28. Raes, A., Detienne, L., Windey, I., & Depaepe, F. (2020) A systematic literature review on synchronous hybrid learning: gaps identified. *Learning Environments Research*, 23(3), 269-290.
- **29.** Butz, N. T., & Stupnisky, R. H. (2016) A mixed methods study of graduate students' self-determined motivation in synchronous hybrid learning environments. *The Internet and Higher Education*, 28, 85-95.
- **30.** Gunawardena, C. N. (1995) Social presence theory and implications for interaction and collaborative learning in computer conferences. *International journal of educational telecommunications*, 1(2), 147-166.
- 31. Crim, S. J. (2006) An examination of social presence in an online learning environment. (Doctoral Dissertation)
- **32.** Tu, C. H., & McIsaac, M. (2002) The relationship of social presence and interaction in online classes. *The American journal of distance education*, 16(3), 131-150.
- **33.** Fritz, S., Speth, C., Barbuto Jr, J. E., & Boren, A. (2004) Exploring relationships between college students' learning styles and motivation. *Psychological reports*, *95*(3), 969-974.
- **34.** Duff, A. (2004) The role of cognitive learning styles in accounting education: developing learning competencies. *Journal of Accounting Education*, 22(1), 29-52.
- **35.** Aguilera-Hermida, A. P. (2020) College students' use and acceptance of emergency online learning due to COVID-19. International Journal of Educational Research Open, 1, 100011.
- **36.** Aquila, M. S. H. (2017) Building the Personal: Instructors' Perspectives of Rapport in Online and Face-to-Face Classes (Doctoral dissertation).
- **37.** Summers, J. J., Gorin, J. S., Beretvas, S. N., & Svinicki, M. D. (2005) Evaluating collaborative learning and community. *The Journal of Experimental Education*, *73*(3), 165-188.
- **38.** Dunlap, J. C., Verma, G., & Johnson, H. L. (2016) Presence + Experience: A framework for the purposeful design of presence in online courses. *TechTrends*, 60(2), 145-151.
- **39.** LaBarbera, R. (2013) The relationship between students perceived sense of connectedness to the instructor and satisfaction in online courses. *Quarterly Review of Distance Education*, 14(4), 209.
- **40.** Hasan, N., & Bao, Y. (2020) Impact of "e-Learning crack-up" perception on psychological distress among college students during COVID-19 pandemic: A mediating role of "fear of academic year loss". *Children and Youth Services Review*, 118, 105355.

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PRESS SUMMARY

The purpose of this study was to examine the relationship between instructor-student rapport and motivation in the context of face-to-face and online classes. Findings revealed a significant positive relationship between instructor-student rapport and student motivation in both online and face-to-face classes. Results suggest students experience more rapport with their instructors during face-to-face classes compared to their online classes, and students reported their motivation is greater during face-to-face classes than in online classes. This study's findings lend further support to research that emphasizes the importance of creating a sense of community in online classes where students feel connected to their instructors and, consequently, motivated to learn.

"The Strong, Silent Type": Analyzing the Portrayal of the Cost of Masculine Gender Performances in The Sopranos

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ABSTRACT

Media portrayals of the "strong, silent type" reinforce the expectation that men should not demonstrate or even acknowledge their emotions. This trope, however, reflects more significant societal norms around masculine practices that can have profoundly negative impacts on individual men as well as those around them.

Emotional compression (or modern stoicism) is fundamentally different from emotional repression. Emotional compression practices can allow men to process their feelings privately and then communicate their feelings clearly without the distortion of uncontrolled bursts of emotion. The treatment of mental health and masculinity in Season 5 of *The Sopranos* "holds up a mirror" to the costs of emotional repression for men as part of masculine gender performances. The show highlights, sometimes quite brutally, the costs of emotional repression to men and the people around them. In doing so, the content of the show implies that therapy could help men learn to face their feelings and alleviate their suffering as well as that of their families, though only if men are willing to face the feelings of vulnerability that come with having emotions.

KEYWORDS

Stoicism; Alexithymia; Hegemonic masculinity; Emotional repression; Mental health; Gender performances

INTRODUCTION

The Sopranos as a series has successfully infiltrated into the zeitgeist of pop culture from its first air date in 1999 to its end date in 2007, with a continued effect long after the show's run ended. From its cult following to its long-lasting resonance with fans and creatives in the entertainment industry, the impact of The Sopranos is extensive, both in terms of its influence on later television programs and its resonance with its audience. The impact has been so long-lasting that Rolling Stone recently ranked the series as the number one greatest television show of all time.¹ A common theme within The Sopranos is the idea of the "American Dream" and its frequently adverse effects on those trying to enact it.² The "American Dream" includes social mobility, consumerism, and materialism.² We focus our analysis on the representation of emotional expression and compression for men. The series shows the effects that trying to enact American hegemonic masculinity characteristics has on their male characters, especially Tony Soprano. We argue that the treatment of mental health and masculinity in Season 5 of The Sopranos "holds up a mirror" to the costs of emotional repression for men as part of performing qualities of hegemonic masculinity.

We first discuss the context of *The Sopranos*, one of HBO's earliest one-hour dramas, with a particular emphasis on its depiction of therapy. We then define and discuss key terms: hegemonic masculinity and alexithymia, as well as comparing emotional repression to emotional compression. Third, we discuss our methodological approach to analyzing the fifth season of *The Sopranos*. Fourth, we present our findings. Finally, we discuss how the series' portrayal of mental health issues and characters' maladaptive behaviors helped to confront the problematic hegemonic masculinity standards depicted in the show. We argue that *The Sopranos* overtly critiques the links between "successful" masculine performances and emotional repression by portraying the negative consequences that occur when men are unable or unwilling to address their emotions.

LITERATURE REVIEW

HBO, The Sopranos, and the depiction of therapy

The Sopranos was one of HBO's first original one-hour dramas and changed the network's whole outlook. HBO was created as a Home Box Office, initially airing movies and live sports events. The network began creating original programming to expand its audience (and paying members). FCC regulations around "decency" are far more lenient for pay cable networks than for broadcast television. On broadcast television, anything considered "indecent" was cut to ensure advertising and the moral

integrity of the audience were not compromised. Cable and pay-cable did not have this responsibility, as they had "more leeway in the area of explicit content and no commercial interruptions." The original programming on HBO had shorter seasons and fewer episodes than broadcasting networks; this gave networks like HBO more money for production. The increased budget for production ensured a realistic, "quality" series for a niche audience of young, urban adults who were expected to handle the graphic content of programs like *The Sopranos* in an "introspective way" and to understand that the series "tackled 'issues' in an insightful manner." The in-depth characterization within the series, created over 100 hours of airtime, allows the audience to understand the characters' behaviors and encourages analysis of why characters are the way that they are, which could not be explored in other, shorter television series.

Understanding the themes and impact of the show extends beyond thinking of it as entertainment. According to Sayre and King (2010), much of what we know about other people comes to us in some form of entertainment. Television reflects American culture, as it "holds up a mirror to society." Television is a form of communication to the audience, not just an art form. Messages in each television series reflect the society communicating the message. David Chase's creation of *The Sopranos* demonstrates this capacity for reflection as the series is in "close synchronicity of the mood and agenda" of 1999 to 2007. Even though the messages may reflect a broader societal mood, not all audiences are going to receive messages the same way or even the way its creators intended. Television serves a "bardic function," as it produces a multitude of complicated meanings varying by audience member; as such, television can prompt conversations about our most traditional views. Television comments on ideological issues in societies but usually never has a full, concise conclusion, which opens audiences up for meaningful conversations about real-world issues of the time.

The Sopranos combined many aspects of television that many still admire and continue to explore. The series combined the "strains" of television styles that already existed (crime programs, soap operas, situational comedy, and therapeutic talk shows) with an overwhelming dark worldview that echoed America's disposition at the time.² For example, the protagonist, Tony Soprano, is deeply flawed but also humanized in such a way that the audience cannot help but root for him, popularizing the antihero as a protagonist on prime-time television. Although Tony Soprano was not the first anti-hero protagonist, and definitely not the last, his character stands out among an array of anti-heroes. In the end, he has no actual redeeming qualities, seen as an "immoral, spiritually bankrupt psychopath who, in the final analysis, is only out for himself." Despite this, many people connected with his character and feel sympathy for him, likely due to their relationship with Tony Soprano from the beginning of the series.⁸

The Sopranos' depiction of its protagonist in therapy sessions is another reason the audience could connect so much with the character, as they could access his deepest, repressed thoughts. Tony Soprano as a character is morally ambiguous, but viewing him in therapy allows a view into this ambiguity, with a discussion of his thoughts, feelings, motives, dreams, and flashbacks that would have otherwise remained invisible. This open portrayal of therapy and how much information it gave the audience about Tony Soprano likely impacted many audience members' views of therapy. Glen Gabbard, professor of psychiatry at Baylor College of Medicine, claimed that the series caused an increase in men seeking out psychotherapy. The portrayal of Tony Soprano discussing his innermost thoughts in therapy allows the audience to identify the reasons behind his panic attacks and outbursts of violent behavior. Many individuals may not have personally experienced therapy or conversations regarding mental health, so portraying these interactions is essential. In particular, U.S. dominant culture encourages all men, regardless of their proximity to the dominant group, to control their emotions and only rely on themselves. Reeking professional help for emotional issues through therapy is non-conforming or resistant to hegemonic masculinity and its associated gender performances. Men, compared to women, are less likely to even "recognize emotional problems when they exist," the first step towards seeking help. A lack of recognition of their emotions is common among male characters in The Sopranos.

Hegemonic masculinity and The Sopranos

Emerging in the 1980s, the term hegemonic masculinity allows researchers to discuss the practices of masculinity that are "the currently most honored way of being a man." Few men, if any, can enact all the characteristics of hegemonic masculinity. Yet, the centrality of hegemonic masculinity to "real" manhood pushes all men to position themselves in relation to this idealized version of masculinity. Although the content of hegemonic masculinity is relational, one can look at the characteristics of masculinity celebrated at a specific time within a particular culture. For example, current American cultural views of hegemonic masculinity advocate "the denial of weakness or vulnerability, emotional and physical control, the appearance of being strong and robust, dismissal of any need for help, a ceaseless interest in sex, the display of aggressive behavior and physical dominance." It is these signifiers of masculinity that individual men must figure out how to engage with and enact.

The hegemonic ideal is ever-changing along with society and circumstance. It depends on society's interpretation of men in relation to women, varying by a wide array of social positions, including race, sexuality, class, national identity, and generation. That said, Schrock and Schwalbe (2009) argue that it is essential to recognize masculinity as a *performance* that asserts claims to

privileges, such as deference to men's preferences and protection from exploitation.¹⁵ Men in different social locations will find some aspects easier to perform than others. For example, in a study of men with licenses to carry concealed guns, Stroud (2012) found that older men turned to carrying a concealed weapon as their capacity to dominate others physically decreased due to age. This alleviated their feelings of vulnerability and allowed them to retain the capacity to elicit fear in others.¹⁶ That is, these men were able to enact a kind of physical dominance and continue to feel like "real" men.

Messerschmidt (1993; 1997) argues that criminal and antisocial behavior offers an array of choices for men to enact masculinity. While Messerschmidt applies this argument to all men (though the type of antisocial behavior varies from group to group), participation in criminal activity is a way for men blocked from legal avenues of economic dominance to demonstrate that they are still men worthy of respect. The men in *The Sopranos* also rely on violence in the illegal market (i.e., "the street") to demonstrate physical dominance and emotional control, as well as deny vulnerability. The cultural setting in the series reflects real societal expectations regarding masculinity. For example, the mafia functions through different power levels, "[a]ll mafias are vertical-and hierarchical- at the family level." There is a ranking system in organized crime; one starts at the bottom of "the family" and works their way up. As seen in *The Sopranos*, the higher ranking one has in the mafia's hierarchy, the more power, money, and respect one receives. And yet, the higher one's ranking within "the family," the higher the potential consequences for stepping out of sync with norms and expectations. *The Sopranos* offers an exaggerated portrayal of the tension many men experience around performing masculinity.

Barnes (2015) analyzes the impact of neoliberalist ideals in *The Sopranos* and *Breaking Bad*, showing the similarities within the shows' frameworks. ¹⁹ The concept of providing for one's family by any means is seen through the main character of each show. This idea is celebrated within hegemonic masculinity in the same way neoliberalist views emphasize profit. Ideals supporting both neoliberal and hegemonically masculine viewpoints underscore the importance of wealth and the individual. These neoliberal ideals appear throughout *The Sopranos*, as characters do anything for financial gain and power, even if it goes against what is morally correct. Collier (1998) suggests that hegemonic masculinity traits in society are associated with characteristics such as "unemotional, independent, non-nurturing, aggressive and dispassionate," which are characteristics with a solid connection to criminal behavior. ^{20, 13} The male characters in *The Sopranos* all strive to perform such aspects of masculinity through their criminal behavior. Their commitment to aggression, emotional repression, and the priority they place on independence (both financial and emotional) are overt.

However, one of the compelling features of *The Sopranos*' storylines is the unflinching portrayal of the costs of these performances. Senior (2017) examines how *The Sopranos* depicts a crisis in masculinity and deconstructs Connell's idea of hegemonic masculinity. Senior argues that the series creates a space for new masculine gender identity through feminist structures encouraging forms of emotion, attachment, and pleasure socially frowned upon otherwise.²¹ Male viewers' attachment to the character of Tony Soprano and the portrayal of his fragile mental state creates an empathetic, feminine connection rather than a narcissistic, masculine one. This depiction of "feminine" behaviors, which benefit Tony Soprano's mental health, further supports the idea that existing masculinity structures hinder emotional growth.²¹ At first glance, Barnes (2015) and Senior (2017) offer competing interpretations of masculine performances in *The Sopranos*. However, we assert that these arguments capture different perspectives on how the male characters enact masculinity. Barnes focuses on how Tony Soprano and other men in the show successfully enact core features of hegemonic masculinity. Senior, on the other hand, highlights the ways the show's plotlines demonstrate how Tony Soprano's misery stems from his inability to understand his emotions.

Emotional compression versus repression

In ancient Greece, the idea of stoicism contained the concept that the morally and intellectually perfect person did not publicly display passionate emotions.²² This does not mean that one does not experience the complete spectrum of emotions, but rather that one guards their passionate feelings from the public sphere to process and experience their emotions fully. Táíwò (2020) refers to this technique as "emotional compression" and suggests that it allows individuals to later communicate their feelings clearly and fully without distorting what they are feeling by publicly portraying one particularly intense emotion.²² This is beneficial, as individuals can process intense, negative emotions, such as anger and aggression, in private rather than through impulsive outbursts. In addition, this could lead to less violence and aggression overall, as individuals can convey their emotions outwardly and verbally, allowing for mutual understanding rather than an altercation. Indeed, Táíwò (2020) argues that emotional compression is potentially pro-social emotional labor for men that scholars often overlook in discussing masculine gender performances.

In modern society, people often confuse the practice of emotional compression with emotional repression. US culture values masculine performances that demonstrate few emotional displays and a capacity for emotional repression as part of "the denial of weakness or vulnerability, emotional and physical control, [and] the appearances of being strong and robust." Psychologists refer to emotional repression and unavailability as alexithymia, which is the impairment of how one recognizes and communicates

one's emotional state.²² Levant (1992) describes men's high incidence of having at least a mild form of alexithymia due to being socialized to be emotionally stoic, as they were encouraged to repress their feelings.²³ A study among individuals experiencing depressive episodes and rates of alexithymia shows that subjects with high rates had trouble with perspective-taking and emotional recognition in others.²⁴ Alexithymia's impairment of emotions also causes individuals to resort to "maladaptive behavioral strategies in response to frustration," such as aggression.²⁵ Put another way, in several studies, violent offenders demonstrate higher levels of alexithymia than non-violent offenders.^{25–27}

Media portrayals of male protagonists, particularly "men of action" ²⁸, frequently embody hegemonic masculinity in ways that men in the real world cannot. ²⁹ These men of action "are strong, silent, and ostentatiously unemotional." ²⁸ The "strong, silent type" is a ubiquitous character that is a trope. ³⁰ Actors such as Gary Cooper, John Wayne, and Clint Eastwood earned fame and professional respect for playing characters who exhibited profound emotional repression. Most cowboys in westerns throughout the early 1900s exemplified the "strong, silent type" trope. ³¹ Several characters on *The Sopranos* overtly reference the "strong, silent type," simultaneously referencing the ability to repress emotional responses as a desirable quality while demonstrating the terrible cost of alexithymia on themselves and the people around them.

In the first episode of the series, Tony Soprano brings up the American actor Gary Cooper, well-known for playing strong, silent types in movies. The "strong, silent type" (and the consequences when real, human men try to embody it) is an overarching theme of the show. Season 5 strongly features this theme. The series' first episode sets the scene for the tensions that come to a head later in the show, as Tony overtly discusses his fears of recognizing his own emotions. In Tony's first visit to his psychiatrist after collapsing due to a panic attack, Dr. Melfi mentions that Tony told his physician that he has been feeling depressed. This obviously makes Tony uncomfortable as he looks around and finds a way to change the subject. Tony brings up Dr. Melfi's Italian heritage and how his mother would have loved it if they got together, using his sexuality as an affirmation of his masculinity to counteract the implied weaknesses of being depressed (i.e., unable to control one's emotional reactions).

Dr. Melfi ignores the comments and again brings up Tony's emotions. Tony, his eyes narrowed and tone increasingly hostile, goes into a monologue about how, nowadays everyone must talk about their problems. He sneers as he says it, implying that all this talking shows weakness. Tony's monologue ends with, "What ever happened to Gary Cooper? The strong, silent type. That was an American. He wasn't in touch with his feelings. He just did what he had to do. See, what they didn't know was once they got Gary Cooper in touch with his feelings that, they wouldn't be able to shut him up! And then it's dysfunction this and dysfunction that!" The very first episode lays out a core tension explored in the show: successful men should not talk about or give in to their feelings, even as Tony sits in therapy doing precisely that. Tony valorizes this emotionally repressed gender performance, even though he also believes even the ideal "strong, silent type" would express a flood of emotions if given a chance to release them.

Emotional compression, however, is not about denying or refusing the existence of feelings. Instead, this concept emphasizes that the processing of emotions is private rather than public. In this way—thinking about stoicism as emotional compression rather than repression—could encourage the perception that using a private therapy space to work through feelings is fundamentally masculine. Tony Soprano seeks out a private space to face his feelings in therapy. Throughout the series, he demonstrates flashes of understanding about the costs of his emotional repression. Despite this, he cannot relinquish his desire to embody the "strong, silent type." Rather than identifying his failure to meet these expectations of masculine performance as a fundamental flaw in the "strong, silent type" itself, he violently lashes out and hurts himself and his loved ones. In doing so, we argue that the show critiques the ties between emotional repression and "successful" masculine performances.

HYPOTHESES

We had three hypotheses prior to conducting our content analysis. Does *The Sopranos*—in its portrayal of its characters—demonstrate: 1) a relationship between hegemonic masculinity and emotional repression, 2) a damaging impact of emotional repression on Tony Soprano in the series, and 3) the damaging effects that Tony Soprano's emotional repression has on the people around him?

METHODOLOGY

The first author watched the entire series and identified main themes focusing on manifest content, which Berg defines as "elements that are physically present and countable." This allowed us to identify themes to analyze in closer detail: masculinity, violence, genetics, repression of emotion, mental health, and therapy. Masculinity, violence, and repression of emotion are central struggles in many of the lives of the male characters. Based on the initial coding, we opted to focus on the entirety of Season 5 because the conflicts of the season—the introduction of an unknown family member, marital stress between Tony and his wife, and Tony's fickle relationship with therapy—highlighted the tensions around emotional displays, control, and masculinity.

Season 5 delves deeper into issues that began developing in earlier seasons, including Tony's aversion to therapy and fear of his own emotions displayed in Season 1, Episode 1 (S1E1). Tony "quit" therapy towards the end of Season 4, citing his lack of progress over the last four years as his reason. He specifically points to his continued lack of impulse control which has contributed to making mistakes at work. In doing so, he glosses over his progress in addressing his depression and panic attacks. Dr. Melfi tells Tony, "now that the panic attacks and the baseline depression have been dealt with, the real work can begin" (S4E11) on finding out who Tony really is behind his mask. After Dr. Melfi says this, Tony states he no longer thinks therapy is working. Truly facing his emotions and the consequences of those emotions is too much for Tony. As he goes through his everyday life without the support of therapy in Season 5, his panic attacks worsen. This process of him quitting and eventually returning to therapy in Season 5 emphasizes the positive differences therapy makes, even without Tony's total commitment to change. Season 5 is, in many ways, the pinnacle of *The Sopranos*' demonstration of the rewards and challenges of therapy.

Once we focused on Season 5, we developed a more detailed coding rubric (see **Table 1**). The first author then engaged in a more fine-grained content analysis that sought to draw out the latent content, the symbolic or deeper structural meaning of the text and scenes.³² This was done by focusing on content that dealt with discussions of masculinity, violence or aggression, repression of emotion, and mental health or therapy. Many scenes fit into multiple categories, relying on unspoken and symbolic connections between unresolved emotions, masculinity, and acts of aggression or violence.

Theme	Looking for
Masculinity	Mention of money, sex, respect, reputation, providing for the family, "real" man, strong silent type
Violence	Any form of aggressive behavior, physical (e.g., hitting, slapping, killing) or verbal (e.g., insulting or threatening someone)
Family/Genetics/Nature	Mention of traits being passed down through the family, statements someone is "just like" another family member
Repression of Emotion	alluding to unresolved issues from the past, lack of self-awareness, inappropriate outbursts, refusal to deal with emotional or relationship problems with other people, signs of depression
Mental Health/Therapy	Mention of psychiatry, therapy, Dr. Melfi, describing people as "insane"/ "crazy," panic attacks

Table 1. Coding Rubric.

RESULTS

Throughout Season 5 of *The Sopranos*, the audience receives more information about Tony Soprano's past and becomes more aware of his unresolved emotional issues. Over the course of this season, it becomes clear that Tony's aggressive behavior and his anxiety attacks stem from a feeling of helplessness that developed from his childhood. A sense of power or control is a demonstration of hegemonic masculinity. The panic attacks that Tony experiences leave him feeling as though he is not in physical or emotional control and that, therefore, he is failing as a man. Tony also suffers from depression, diagnosed by his psychiatrist Dr. Melfi, which often makes people feel powerless and out of control, prompting more feelings of failure. These underlying feelings, accompanied by his responsibilities as the boss of both his traditional family and organized crime family, cause emotional conflict. Tony demonstrates certain traits resembling alexithymia, which frequently co-occurs with depression. The inability to deal with his emotions causes him to resort to negative behaviors. Tony's emotional conflicts and feelings of failure as a man cause him to lash out aggressively at those closest to him, both verbally and physically.

The heightened expectations of men's behavior in the mafia are comparable to the hegemonic masculinity expectations of men in society. The inability to live up to these expectations creates frustration, sadness, and resentment that the men in *The Sopranos* do not know how to recognize or deal with healthily, resulting in outbursts of aggression and violence. The men in *The Sopranos* demonstrate characteristics of alexithymia and emotional repression rather than emotional compression. The plotlines of the show draw on the consequences of that emotional repression and, in doing so, demonstrate the negative impact of emotional repression on the male characters and the people around them.

The Effect of Tony Soprano's Behavior on Himself

Tony Soprano has hegemonic masculine ideals pushed at him from all directions. His father and uncle were also in the mafia and taught Tony the masculine expectations he must embody to be successful as a member of organized crime. Tony learns that repressing his feelings and denying weakness or vulnerability to anyone (even those closest to him) are core facets of respected masculine gender performances. He also learns acceptable outlets for his feelings from his family: eating, enacting violence, and pursuing sexual conquests. All three of these "acceptable" outlets cause harm to himself and others.

Tony demonstrates his aversion to therapy in S5E4, where the "strong, silent type" is again overtly referenced. While discussing AJ (Tony's son)'s future with his guidance counselor, the possibility of AJ seeing a psychologist arises. The guidance counselor asks Tony whether he approves of therapy. Despite his experiences with therapy's positive impact, Tony cannot express support, stating, "People use it as a crutch. And I always wonder what happened to Gary Cooper, the strong silent type." This ideal of the "strong, silent type" echoes throughout the series, as Tony demonstrates how deeply rooted he believes that the denial of weakness or vulnerability is integral to "real" masculinity, even as the burden of repressing his feelings eats away at his mental stability.

Therapy and counseling do not fit into the societal gender roles assigned to men, pushing them to view these resources as negative and stigmatize themselves and others for needing help. This stigmatization only further encourages alexithymia behaviors seen in these characters, as they refuse to accept the support they need to understand and process emotions. Tony's attitude towards therapy reflects this, both in his refusal to let his son participate in therapy and his own conflicted feelings about using therapy himself.

Tony's panic attacks are a negative consequence of his inability to express his feelings. Anything that causes him to access repressed and unresolved emotions from his past trigger the attacks. These emotions create such anxiety that his body goes into panic mode; he becomes breathless, starts sweating, his vision blurs, and he occasionally even loses consciousness. The panic attacks seen in Season 5 reflect unresolved emotions around events just before his cousin was arrested, something that Tony does not like to speak about. The feelings are so powerful that the mere mention of the word "cousin" prompts a panic attack in S5E4. Carmela is chatting about her cousin and Tony must sit down as he starts to have shortness of breath.

Tony Blundetto, Tony Soprano's cousin, has been in prison for the last 17 years for a hijacking gone wrong; he returns home in Season 5. Tony S. and Tony B. grew up together, along with Christopher Moltisanti, and were close in their youth. Both of them were supposed to participate in the hijacking that ultimately sent Tony B. to prison. At the beginning of Season 5, the audience learns that Tony S. was in the hospital during the hijacking because he got mugged by a group of men. However, in S5E9, Dr. Melfi points out that Tony's recent panic attacks have all been brought on by conversations mentioning cousins, demonstrating the long-lasting effects of Tony's unresolved emotions from the past.

While almost having a panic attack during the discussion, Tony admits to Dr. Melfi that he was in the hospital that night because he got into an argument with his mother, had a panic attack, passed out, and hit his head, requiring stitches. Out of embarrassment and shame of his "weakness," Tony concocted a false story of what happened, causing him to repress his guilt about his cousin going to prison while he did not. This burying of emotions for fifteen years, encouraged by masculine performances to remain "strong and silent," have contributed to severe panic attacks that affect the quality and safety of Tony's life. Tony S.'s guilt, both at having had a panic attack and then lying about it, casts a shadow on his relationship with his cousin. Tony S. is certain that his cousin resents the fact that Tony S. should have been at the hijacking. For Tony S., the possibility that he could have helped his cousin avoid prison if only he had not been "weak" haunts his current interactions with his cousin. The two of them butt heads over the course of Season 5, and instead of Tony S. reuniting with someone he was close to in his youth, he collects yet another enemy.

Tony's isolation is another negative consequence of his emotional repression. His relationships with his wife and children suffer because he cannot express his feelings to anyone. His wife, tired of Tony's infidelity and emotional unavailability, asked for a divorce at the end of Season 4. In Season 5, Tony lives in his mother's old house. Tony's relationship with his mother was deeply conflicted, something Tony has avoided addressing in therapy. Indeed, Tony's most profound and most painful emotions have

roots in his dysfunctional relationship with his mother. Living in a house full of echoes of his late mother, a woman Tony loved and hated in equal measures, Tony receives almost no visitors.

Tony's relationship with his nephew, Christopher Molitsanti, also demonstrates Tony's isolation and inability to connect meaningfully with the people he cares most about. Christopher struggles with addiction to heroin. Christopher went through an intervention and was sent to a rehab facility in episode 10 of Season 4 (an episode entitled "The Strong, Silent Type"). When he returns in Season 5, he is clean. He has done some preliminary work to acknowledge and address his emotions and the impact those emotions had on his addiction, something that Tony cannot wrap his head around. The show's creators use these storylines and characters to highlight the ongoing problems Tony's emotional repression creates.

Tony's "right hand" man. Season 5 focuses on the yawning distance between these two characters. Overall, Tony and Christopher are in very different places in Season 5. Tony, now living on his own, is drinking, partying, and eating more than he had been while living with Carmela. Christopher, however, is sober, focusing on himself and his future. Tony swings back and forth between shaming Christopher for his addiction and then shaming him for his sobriety, which takes a toll on Christopher. Tony goes back and forth with himself, shaming himself for his "weakness" and mental health struggles and then shaming himself for attending therapy which helps him with these struggles. Tony's disconnect from this new, sober Christopher is seen in S5E10. While upstate with his cousin and nephew, Tony encourages Christopher to have a drink after stating he is sick of hearing his hardships about his sobriety. This comment sticks with Christopher; on his drive back to New Jersey afterward, Christopher tears up. Christopher references this moment again in S5E12 while talking to his fiancée, Adriana. Christopher is upset about Tony's repeated digs regarding his sobriety. He expresses his frustration and resentment about Tony to Adriana, stating he is done with his uncle, and that he could take Tony out in a second.

This evolution of Christopher is integral in critiquing Tony's dated, damaging ties to gender and emotional expression. Christopher's sobriety is important as it resembles Tony's mental health journey. When Christopher is struggling, it provides an opportunity for Tony to provide support to someone he loves. However, Tony sees this vulnerability in Christopher as a weakness, the same way he sees it in himself. It is a threat to manhood and Christopher's duty as his soldier. Tony's reaction is to go on the attack, to force Christopher to repress his feelings as a real man should, instead of helping Christopher through emotional connection. This outlook destroys Christopher and Tony's relationship, leaving Tony isolated at the top.

As Tony chooses to avoid the steps towards emotional maturity he discovers through therapy, and potentially the enactment of compression rather than repression, the negative consequences become more apparent. Tony's overeating is a negative consequence of his inability to express his feelings. In S5E2, Tony's weight plays a role in the tensions between himself and his cousin. Tony B. cracks jokes regularly about Tony's weight. Tony laughs them off to Tony B.'s face, but later, Tony wakes up from a night of drinking alone in his mother's old home. After drunk dialing Tony B. the night before, Tony S. looks in the mirror and takes off his shirt, squeezing and examining his gut. Finally, he examines himself in the mirror, looking disappointed. This shows Tony is aware of his unhealthy habits with food and their effect on his body and health. However, he can't help but continue to use food to cope with his inability to express his emotions.

Effect of Tony Soprano's Behavior on his Families

Tony returns to therapy in S5E5 to discuss his attraction toward his nephew's fiancée, Adriana, with Dr. Melfi. It is a moment where the audience can see Tony's progress over the four years of therapy. He seeks to discuss his impulses rather than instantly acting on them. Tony admits that it would "kill" Christopher if he ever had sex with Adriana. Still, instead of acknowledging that he loves Christopher, Tony points to his years of training his nephew to be his number two as the reason this betrayal would be a disaster. Despite this twisted view, Dr. Melfi points to his return to therapy to avoid destructive behavior as a milestone. Dr. Melfi advises Tony to be honest with himself, stating that if he believes he cannot control his urges, he should set some boundaries and stay away from Adriana.

The next time Tony sees Adriana, they both deliberately try not to be alone together. By the night's end, however, Tony offers to drive her to score some cocaine. Unfortunately, while driving, Tony swerves to avoid a raccoon and crashes the car early in the morning. Although Tony did not betray Christopher, he did not follow through with his plan to maintain boundaries. If they had not gotten in a car crash, it is unclear whether Tony and Adriana would have avoided acting on their attraction.

The news of the crash spreads amongst the family, along with rumors of a sexual relationship between Adriana and Tony. By the time these rumors get to Christopher, people are saying Adriana was giving Tony a blowjob and that this was the reason for the car crash. The rumors leave Christopher distraught and paranoid. Tony's years of sexual infidelity make it difficult for Christopher to believe that nothing happened between his fiancée and his uncle. Christopher loses his temper, assaults Adriana, and then kicks

her out of their house. He first looks for cocaine in Adriana's purse and, when he cannot find it, drowns his feelings of betrayal in alcohol. Christopher drives drunk to Bada Bing, the strip club owned by Tony's associate, and shoots at Tony's car parked out front. He runs out of bullets and enters the bar, where Tony is seated upstairs. Christopher aims the gun at Tony and is immediately disarmed by security guards. Christopher screams, "you're lucky I ran out of loads," as he is dragged outside. This conflict between Tony and Christopher is the first of many, leading to the death of their relationship and, ultimately, Tony's murder of Christopher.

Tony's relationship with Christopher is entangled with Christopher's sobriety. Tony was the one who sent Christopher away to rehab and then is the leading cause of Christopher's first relapse. Tony provides some support and responsibility for Christopher's relapse this time by sparing his life and allowing Christopher to hear from the doctor that nothing could have occurred between him and Adriana due to her body position during the crash. To face the rumors head-on, Tony convinces Carmela to attend dinner at Vesuvio's with him, Adriana, and Christopher, proving to other members of the "family" that all issues are resolved. Christopher, however, still feels humiliated.

Tony's support and ability to connect with Christopher are due to his attendance of therapy within this episode. Since Tony was first able to discuss his feelings in therapy, he had a better understanding and control over his emotions. As a result, he could connect with Christopher during his emotional crisis, empathizing with him rather than becoming angry with him. However, this support is not seen in future conflicts between Tony and Christopher, despite Tony's understanding of how fragile Christopher's sobriety is, especially when it comes to issues regarding Adriana.

In S5E10, Tony lashes out at Christopher when the two of them are having dinner with Tony Blundetto. Tony brings up Christopher's sobriety, poking fun at it and eventually encouraging him to have a drink, stating he is "driving everybody crazy" with his 12-step program. Tony is aware of Christopher's struggles with sobriety as he saw how fragile it was in Episode 5 but still unleashes this verbal aggression. Despite his own mental health struggles, Tony cannot let go of the belief that asking for help is a weakness. Although he later apologizes and admits that Christopher is doing the right thing, Tony takes this moment to align with Tony B., who, at that point in the series, is more effectively displaying the ideals of hegemonic masculinity that Tony values. Tony B. seems self-sufficient and strong, while Christopher must rely on others' support to stay clean. This specific example is interesting, as it deals with addiction when discussing self-betterment. Rehabilitation programs work best if the individual accepts their problems, legitimately wants to change, and works to do so. The same can be said regarding traditional therapy, emphasized in the series with Tony Soprano. Tony is in therapy but can never seem to show a changed mindset in the way Christopher does (at least, for a while). Tony represses his frustration and helplessness, redirecting those emotions into anger and resentment.

In S5E12, Christopher's sobriety falters again, and Christopher reaches out to Tony for support. However, this time Tony's response reflects his inability to deal with guilt and sadness. Adriana La Cerva's involvement with the FBI ends as she finally tells Christopher that she has been an informant for the past couple of months. Ultimately, Christopher exposes the truth to Tony, who then hires a hit on Adriana to protect his work "family." After Adriana is murdered, Christopher begins using heroin again to cope with the loss of his fiancée and his role in her death. Tony finds Christopher in his office watching television, where the two demonstrate their grief over losing someone they both cared about through self-destruction and violence.

In the scene, Tony walks into his office, rubbing his face and letting out a deep sigh when he sees Christopher. He asks if Christopher is alright, though he already knows something is wrong. As Christopher talks, Tony's whole body tightens in rage.

Tony Soprano: The fuck is wrong with you?

Christopher: I snorted a little H. I know... but I can't stand the pain... I loved her.

Tony Soprano: Fucking pain, huh... you think you're alone in this!

Tony starts by breathing deeply, perhaps trying to control his anger, but as Christopher continues talking about his feelings about Adriana and her death, Tony's brow furrows, and his teeth clench before he violently attacks Christopher, knocking him down and repeatedly kicking his nephew while Christopher cries out in pain. Tony's alexithymia characteristics mean he cannot effectively pursue healthy ways to deal with his anger, fear, frustration, and anxiety. There is a sort of jealousy there, as Christopher can release this emotion and fall apart; however, Tony cannot since he is the boss and must show emotional control and strength. Tony pushes any emotion other than anger to the side to remain able to do business. Tony's statement, "you think you're alone in this," inserts an enormous amount of unaddressed emotional suffering into his assault on his nephew. This unaddressed suffering directly linked to Tony's violent outburst against someone he truly cares about shows the costs of these masculine ideals.

Tony's inability to express his sadness and guilt about Adriana's death and to demonstrate genuine concern and care for his nephew are a result of his years of emotional repression, which only creates further emotional issues that need to be resolved. Tony's refusal to support Christopher, punishing him as he reaches out for help, kills the possibility of Christopher's future

recovery. Tony's actions communicate to Christopher that he will not encourage this behavior. Just like Tony, Christopher must bottle up his emotions, stay "strong," and deal with things on his own, which does not align with successful sobriety. These scenes lay the groundwork for Tony's eventual murder of his nephew in Season 6.

Tony's refusal to deal with his feelings from his past also contribute to Tony Soprano's eventual murder of his cousin, Tony Blundetto. In S5E9, Tony has a breakthrough in therapy regarding his panic attacks and his guilt about his cousin's arrest and imprisonment. The embarrassment and shame of his weakness, and the guilt that he might have contributed to his cousin going to prison, has followed Tony into adulthood. This discovery gives Tony power as he has found a trigger of his panic attacks and can now address the emotional issues head-on. Dr. Melfi encourages Tony to come clean to Tony B. Tony, however, cannot make himself do it. After years of emotional repression, the thought of confessing the truth and communicating his emotions is foreign to Tony.

This knowledge that his panic attacks are an expression of guilt and embarrassment about the events around the ill-fated hijacking seventeen years ago weighs heavily on Tony. He would also have to admit weakness both then and now to admit the truth. His inability to talk through the issue with Tony B. creates intense anxiety for Tony, which he deals with through violent and aggressive behavior. For example, in S5E10, Tony loses control while having a casual discussion with one of the bartenders at Bada Bing. In the conversation, Tony focuses on a threat he cannot control to alleviate his anxiety: the proximity of the airport to their homes and businesses and the potential threat of a terrorist attack. The bartender shrugs and suggests that the solution is to "live for today." The answer enrages Tony, who proceeds to violently assault the bartender, throwing glasses at him and bashing him over the head with anything he can grab.

The relationship between Tony Soprano and Tony B. sours over the course of Season 5. This is because of Tony Blundetto's struggles to lead a "straight and legal" life and Tony Soprano's continued avoidance of admitting what happened seventeen years ago. Tony Soprano only ever manages to discuss what happened the night of the hijacking with his cousin when he already knows he must execute his cousin. Not long after returning home from prison, Tony Blundetto returned to organized crime, killed someone, and angered the head of another mafia family. The tension between Tony is finally resolved in S5E12 when Tony admits the truth to Tony B., but it is too late. The damage of the secret is irreversible. Tony Soprano murders his cousin with a shotgun, seeking to spare his cousin's torture at the hands of other mafia members.

There are other examples of the negative impact of Tony's emotional repression: the tumultuous relationship with his wife, his tense relationship with each of his children, and his resentment-infused relationship with his sister. However, we focused on Christopher, Adriana, and Tony B. because in each of these instances, Tony Soprano ends up murdering the sources of his "weakness." Though he struggles to do so, Tony is unable to face, process, and express any feelings beyond anger. Being the boss of both families, his mafia and traditional family, he bottles up his emotions and, as Christopher says in S5E10, eats his feelings instead. Even Christopher, however, does not seem to see the link between Tony's emotional repression and his aggressive and violent outbursts. Tony's use of therapy throughout the show offers the audience a clear view into Tony's struggle to incorporate what he learns in therapy about his emotions and his desire to be strong and silent. Watching him struggle to enact what he learns in therapy highlights the costs of his failure: misery and death.

DISCUSSION

The Sopranos' portrayal of Tony Soprano creates an honest depiction of a man struggling with how to deal with his emotions while still trying to successfully fulfill his perceived role as a man in his family and his career. The thematic content of the series shows the level of masculinity expectations in society and the stigma placed on men for accessing mental health help. These two pressures strongly affect Tony Soprano, as he is held to a standard of masculinity as the boss of both his "families," and because he suffers from panic attacks and attends therapy to treat them. The contradiction between the external (i.e., being "strong and silent") and the internal (i.e., being truly stoic requires emotional vulnerability and expression) form a dominant theme of *The Sopranos*.

The series shows a level of connection between emotional repression, the trait of alexithymia, and aggressive behavior. The men throughout the series, particularly Tony Soprano, repress their emotions to maintain respect in the organized crime world. This affects their personal lives and the people around them. A pattern emerges as repressed emotions are brought to the surface and ignored repeatedly. Characters deal with these repressed feelings through aggressive or violent behavior that helps them avoid the frightening sense of vulnerability. Many of the characters lack the skills true emotional compression requires. The masculine expectations of men to behave a certain way to be respected encourages negative alexithymia behaviors, which lead to negative reactive behaviors when unprocessed emotions unexpectedly come to the surface.

The focus in Season 5 is Tony Soprano's unresolved emotions from his past and the consequences of that lack of resolution on himself and those around him. Tony's impulsive outbursts of aggression create problems for him at work and home. The theme of therapy throughout the series explores Tony trying to cope with his mental state and being unable to commit fully to the process because letting go of his idea of traditional masculinity is too frightening. For Tony Soprano, his role in the mafia does make this fear more realistic. Other men's perception that he is not manly enough could cause them to end his life. Yet, many men feel this fear even without the literal threat of murder. As Tony resists dealing with his emotions, his outbursts and panic attacks become more apparent, allowing the audience to see how unreasonable masculinity expectations and repressed emotions have profoundly negative impacts on an individual's mental state and behavior.

The series' exploration of mental health issues on such a large platform helped to defy the masculinity standards represented in the show. Overall, *The Sopranos'* exploration of mental health and masculinity brings awareness to the costs of men's emotional repression due to society's masculinity expectations. The outbursts of violence and aggression seen throughout the series are almost as common as the avoidance of one's emotional conflicts. The series ends with a cut to a black screen in the middle of a scene, giving the audience no resolution or closure. *The Sopranos'* plotlines do not offer simple or easy answers. Embracing therapy could have potentially helped Tony Soprano avoid harming the people around him. The audience will never know, however, because Tony pulled back from his emotions in fear and could not escape the destructive allure of the "strong and silent" masculine gender performance.

CONCLUSIONS

The most obvious place to start with future research is to expand the detailed coding in this article to the rest of the show's seasons. One of the main arguments of this article is that emotional repression plays a significant part in impulsive aggressive behaviors. Interesting patterns would emerge with more story arcs included in the analysis. This paper primarily focused on Tony Soprano and a few other key male characters. Future analysis could address more characters' approaches to dealing with difficult emotions. Another potential direction for future research is considering other series that feature therapy and mental health. For example, both *Tell Me You Love Me* and *In Treatment* are HBO series that deal with therapy as a central part of their storylines. How do these series deal with masculinity, emotional suppression, and aggression? It would be intriguing to see if *The Sopranos*' exploration of these topics is continued in the subscription network's later series, and if ideas surrounding these topics have evolved over time.

REFERENCES

- 1. Rolling Stone, The 100 Greatest TV Shows of All Time, https://www.rollingstone.com/tv-movies/tv-movie-lists/best-tv-shows-of-all-time-1234598313/jeopardy-2-1234599051/ (accessed Dec 2022)
- 2. Edgerton, G. R. (2013) The Sopranos (TV Milestones Series)., 1–100, Wayne State University Press, Detroit, Michigan.
- 3. Jaramillo, D. L. (2002) The family racket: AOL time warner, HBO, *The Sopranos*, and the construction of a quality brand, *Journal of Communications Inquiry* 26:1, 59–75. https://doi.org/10.1177/0196859902026001005
- 4. Williams, B. (1994) North to the future: Northern exposure and quality television, in *Television: The Critical View* 5th ed., edited by Horace Newcomb, Oxford University Press, New York.
- 5. Sayre, S. and King, C. (2010) Entertainment and Society: Influences, Impacts, and Innovations, 315, Taylor & Francis Group.
- 6. Lule, J. (2018) Understanding Media and Culture: an Introduction to Mass Communication, 3858–389, FlatWorld.
- 7. Newcomb, H. and Hirsch, P.M. (1983) Television as a cultural forum, *Quarterly Review of Film Studies*, 561–573. https://doi.org/10.1080/10509208309361170
- 8. Vaage, M.B. (2013) Fictional reliefs and reality checks, Screen 54:2, 218–237. https://doi.org/10.1093/screen/hjt004
- 9. Canet, F. (2019) More therapy with Dr. Melfi (the character who guides viewer engagement with Tony Soprano): Relationship arcs in serial antihero narratives, *Journal of Screenwriting* 10:1, 97–112. https://doi.org/10.1386/josc.10.1.97_1
- Wynn, N.A. (2004) Counselling the mafia: "The Sopranos", Journal of American Studies 38.1, 127–132. DOI: 10.1017/S0021875804007947
- 11. Mahalik, J.R., Locke, B.D., Ludlow, L.H., Diemer, M.A., Gottfried, M., Scott, R.P.J., and Freitas. G. (2003) Development of the conformity to masculine norms inventory, *Psychology of Men and Masculinity* 4:1, 3–25. https://doi.org/10.1037/1524-9220.4.1.3
- **12.** Kessler, R.C., Brown, R.L., Broman, C.L. (1981) Sex differences in psychiatric help-seeking: Evidence from four large-scale surveys, *Journal of Health and Social Behavior* 22, 49–64. https://doi.org/10.2307/2136367
- 13. Connell, R.W and Messerschmidt, J.W. (2005) Hegemonic masculinity: rethinking the concept, *Gender & Society* 19.6, 829–859. DOI: 10.1177/0891243205278639
- Courtenay, W.H. (2000) Constructions of masculinity and their influence on men's well-being: a theory of gender and health, Social Science & Medicine 50.10, 1385–1401.
- **15.** Schrock, D. and Schwalbe, M. (2009) Men, masculinity, and manhood acts, *Annual Review of Sociology* 35, 277–295. 10.1146/annurev-soc-070308-115933

- 16. Stroud, A. (2012) Good guys with guns. Gender & Society, 26:2, 216–38. https://doi.org/10.1177/0891243211434612
- 17. Messerschmidt, J.W. (1993) Masculinities and Crime: Critique and Reconceptualization of Theory, Lanham, Md: Rowman & Littlefield.
- 18. Catino, M. (2014) How do mafias organize? Conflict and violence in three mafia organizations, Archives Européennes de Sociologie. European Journal of Sociology 55.2, 183. DOI: 10.1017/S0003975614000095
- 19. Barnes, C. (2015) Ungoverned Masculinities: Gendered Discourses of Neoliberalism in The Sopranos and Breaking Bad M.A. Thesis, Department of Communication and Journalism, The University of New Mexico, 5. https://digitalrepository.unm.edu/cj_etds/85/
- **20.** Collier, R. (2002) Masculinities, Sage Journals 36.3, 737–742.
- 21. Senior, J. (2017) Walk Like a Man: Hegemonic Masculinity and Un-Made Men in 'The Sopranos' M.A. Thesis, Department of English Literature, University of Huddersfield, 9-16. http://eprints.hud.ac.uk/id/eprint/34156/
- 22. Táíwò, O.O. (2020) Stoicism (as emotional compression) is emotional labor, Feminist Philosophy Quarterly 6 (2) Article 4, 5–9. https://doi.org/10.5206/fpq/2020.2.8217
- 23. Levant, R.F. (1992) Toward the reconstruction of masculinity, Journal of Family Psychology 5.3-4, 388.
- 24. Banzhaf, C, Hoffmann, F, Kanske, P, Fan, Y, Walter, H, Spengler, S, Schreiter, S, Singer T, and Bermpohl, F. (2018) Interacting and dissociable effects of alexithymia and depression on empathy, *Psychiatry Research* 270, 631–638. https://doi.org/10.1016/j.psychres.2018.10.045
- 25. Garofalo, C, Velotti, P and Zavattini, G.C. (2018) Emotion regulation and aggression: the incremental contribution of alexithymia, impulsivity, and emotion dysregulation facets, *Psychology of Violence* 8.4, 470–483. http://dx.doi.org/10.1037/vio0000141
- **26.** Manninen, M, Therman, S, Suvisaari, J, Ebeling, H, Moilanen, I, Huttunen, M, and Joukamaa, M. (2011) Alexithymia is common among adolescents with severe disruptive behavior, *The Journal of Nervous and Mental Disease* 199.7, 506–509. doi:10.1097/NMD.0b013e3182214281
- 27. Teten, A.L, Miller, L.A, Bailey, S.D, Dunn, N.J, and Kent, T.A. (2008) Empathic deficits and alexithymia in trauma-related impulsive aggression, *Behavioral Sciences & The Law* 26.6, 823–832. (www.interscience.wiley.com) DOI: 10.1002/bsl.843
- 28. Russo, V. (1987) The Celluloid Closet: Homosexuality in the Movies (Rev. Ed.) Harper & Row, New York.
- 29. Wood, J.T. (2009) Gendered Lives: Communication, Gender, and Culture (8th Ed) Wadsworth.
- **30.** Hobbs, T. (2017) Does the 'strong, silent type' still exist?, *AnotherMan*, Another Man Publishing Ltd. https://www.anothermanmag.com (Accessed Mar 2021)
- 31. Rainey, B. (2004) The Strong, Silent Type: over 100 Screen Cowboys, 1903-1930, McFarland & Co.
- 32. Berg, B.L. (1989) Qualitative Research Methods for the Social Sciences, 308, Boston: Allyn and Bacon.
- **33.** Warren, L.W. (1983) Male intolerance of depression: a review with implications for psychotherapy, *Clinical Psychology* Review 3.2, 147–156. https://doi.org/10.1016/0272-7358(83)90009-0

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PRESS SUMMARY

Media portrayals of the "strong, silent type" reinforce the expectation that men should not demonstrate or even acknowledge their emotions. This idea reflects common societal standards regarding masculinity, which can seriously impact individual men and those around them. Táíwò (2020) argues that emotional compression (or stoicism) fundamentally differs from emotional repression. Emotional compression allows individuals to process their feelings privately and then communicate them clearly and thoroughly without distorting, uncontrolled bursts of emotion. The treatment of mental health and masculinity in Season 5 of *The Sopranos* "holds up a mirror" to the costs of emotional repression for men as part of masculine gender performances. The show highlights, sometimes quite brutally, the costs of emotional repression to men and the people around them. In doing so, the content of the show implies that therapy could help men learn to face their feelings and alleviate their suffering and their families, though only if men are willing to face the feelings of vulnerability that come with having emotions.

A Review of the Effect of Estrogen on Immune Efficacy in Zebrafish (*Danio rerio*) with Comparisons to Human and Murine Homologs

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ABSTRACT

A review was conducted on current research surrounding the effect of estrogen, and the estrogen receptor, on immune development. Estrogen can regulate many processes and genes throughout immune development, from modulating complement activation and regulating genes crucial for hematopoiesis, to elevating toll-like receptor gene expression. Estrogen has also been shown to have a pronounced effect on regulating certain cancers through inducing macrophage infiltration. It has also been demonstrated to play an important role in the regulation of microRNAs that are important for proper immune development. A greater understanding of this hormone's effect gained through the zebrafish model can lead to the development of better practices to improve both human and ecological health. Contemporary reviews typically examine the effect of estrogen-like compounds (oftentimes referred to as estrogenic endocrine disrupting compounds) on a sequestered part of immune system development. A distinct lack of cohesion exists in combining contemporary and past reports of the effects of estrogen on various aspects of immune system development in zebrafish. This review serves to fill that gap in knowledge, and to provide a gateway for other researchers interested in this topic.

KEYWORDS

Zebrafish; Immune development; Zebrafish immunology; Estrogen; Estrogen receptor; Autoimmunity; Altered signaling; Hematopoiesis

INTRODUCTION

Estrogen is an important hormone for development in vertebrate species, including zebrafish (*Danio rerio*), an increasingly popular animal model for its ease of care, transparency during development, and the similarity of many of its systems to humans. While estrogen activity is primarily associated with development of sexual organs and reproduction within female members of a species, it has an effect in a variety of systems, both during initial development and throughout the life of the organism. As with other hormones, estrogen operates within tightly controlled levels, with significant variation in these levels resulting in a variety of disorders. For example, estrogen deficiency can result in loss of bone density, metabolic disorders resulting in increased weight gain and LDL cholesterol, and fertility problems in adults. An overabundance of estrogen can be just as damaging, leading to a variety of conditions including breast tumor development, with the increased expression of this hormone in females being correlated with higher rates of autoimmunity.

There have been two primary estrogen receptor subtypes identified in zebrafish, the Esr α and Esr β (also known as the ER α and ER β , respectively). Within the Esr β subtype, there are two additional receptors, and these are the Esr2a, and the Esr2b receptors. There also exists the somewhat enigmatic GPER, or G protein-coupled estrogen receptor, which is a recently discovered membrane bound receptor that may regulate innate immunity. These receptors have been found in a variety of tissues within the zebrafish, including the liver and kidney. Prior research has determined that the human ER β is homologous to zebrafish ER β a, in both function and structure. The human ER α has also been confirmed to be orthologous to the zebrafish's ER α .

This study will explain where these estrogen receptors and estrogen signaling might have the strongest effect in the zebrafish model pertaining to immune development, based on contemporary research. Due to the increased prevalence of estrogenic chemicals that can signal through the estrogen receptor, such as bisphenols, it is essential that the research community investigates the mechanism of estrogen- and estrogenic-induced immune function manipulation, in aquatic and terrestrial wildlife as well as human populations. ^{13, 14} Different chemicals can bind to these estrogen receptors other than the hormone after which it is named. For example, BPA and some of its analogs can interfere with estrogen signaling. ¹⁵ These chemicals, which belong to a class of

substances called estrogen disrupting chemicals (EDCs), can also include dichlorodiphenyltrichloroethane (DDT), dioxin, phthalate esters, atrazine, etc. 16

Female zebrafish can typically respond to estrogens through the expression of biomarkers such as vitellogenin, which is a yolk precursor protein.¹⁷ This expression is atypical in males but can occur in hepatocytes after exposure to both natural and synthetic estrogens.^{17, 18} Estrogens can also lead to reduced gonadal maturation and even reverse sex differentiation in males.¹⁹ Published research from a variety of peer-reviewed sources was used to construct this review. Topics chosen for coverage in this review ranged from estrogen's effect on complement activation, toll-like receptor signaling, thymic growth, notch signaling, and cytokines, to innate cells and micro RNAs.

Complement

Complement activation is a crucial part of humoral innate immunity. It is a series of three unique pathways of plasma proteins that form a membrane attack complex (MAC) once activated by pathogens; in turn, it opsonizes the pathogens to neutralize them, or aids in the recruitment of specialized phagocytic cells such as macrophages.²⁰ Therefore, reducing its effectiveness in some capacity from excess estrogen exposure can cause serious issues for immune development, with the results being potentially fatal. Recent research has demonstrated that zebrafish larval exposure to elevated 17 β-estradiol (or E2) levels can cause a toxic accumulation of factor H, which is a regulatory protein of the alternative pathway of the complement system that is responsible for ensuring the MAC does not assemble on self-cells.^{21, 22}

Estradiol is an important estrogenic hormone that plays a key role in the proper development of multiple functional systems throughout the body, and serves as an agonist of the two classical subclasses of estrogen receptors. ^{23, 24} When challenged by *C. albicans* with and without E2 exposure, it was determined that zebrafish exposed to 1μM of E2 yielded a 63% decrease in the survival rate post-infection. ²¹ While C3b, an important complement factor, was able to bind to the fungal cell surfaces, the heightened factor H recruitment, effectively prevented phagocytosis by inhibiting the membrane attack complex from forming. ²¹ The usual function of factor H is to regulate complement activation through accelerating the degradation of C3b on either the surface of targeted cells or in the fluid-phase. ²⁵ As C3b is an integral component of both the alternative and classical pathways, this allows factor H to prevent two of the three pathways that are available (although it is generally associated with the alternative pathway). ^{21, 26} Factor H is essential in regulating a pro-inflammatory response in the body; in fact, its very absence or downregulation has been linked to diseases such as hemolytic uremic syndrome or glomerulonephritis. ^{27, 28} In the case of estrogen-induced overexpression, however, it can prevent the formation of the MAC and halt two of the three complement pathways altogether; this in turn can lead to higher infection susceptibility and elevated fatality rates in zebrafish. ²¹ Future research could be performed on other regulatory components of the complement system that work in conjunction with Factor H, such as complement receptor type 1 (CR1) or decay accelerating factor (DAF), to further establish if estrogen can cause other aberrant effects during development of the zebrafish innate immunity.

Toll-like Receptors

Estrogen is also involved in the expression and regulation of the transmembrane toll-like receptors (TLRs) in zebrafish. TLRs are one of multiple subsets of PRRs, or pattern recognition receptors, which are used for the early detection of foreign substances through its recognition of distinctly non-self molecules, such as double stranded RNA (dsRNA), flagellin, or even lipopolysaccharide (LPS).²⁰ TLR's are found on a variety of cell types, from keratinocytes and innate immune cells such as dendritic cells, to adaptive immune cells like B lymphocytes; this early TLR-mediated detection is vital for downstream cytokine signaling to occur, which in turn recruit neutrophils, macrophages, and dendritic cells in the early immune response.²⁹⁻³¹

Male zebrafish embryos treated with high levels of E2 were associated with an elevated expression of genes coding for Esr and TLRs, which resulted in additional nuclear factor kappa B (NF-μB) expression.³² When TLRs detect the presence of a pathogen associated molecular pattern (PAMP), such as the bacterial cell wall component LPS, this triggers a complex cascade within cells that eventually results in the release of NF-μB.^{33, 34} NF-μB is a regulatory transcription factor that, once activated, ends a cascade that causes the nuclear transcription of a variety of pro-inflammatory cytokines and adhesion molecules.³⁵ See **Figure 1**.

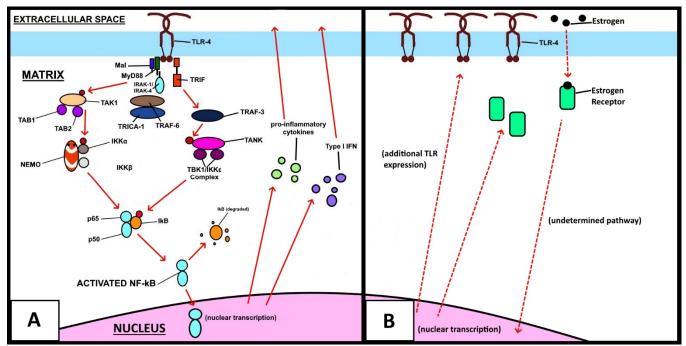


Figure 1. (A) TLR signaling. The figure above details both the canonical NF-xB pathway through MyD88, and the non-canonical pathway through TRIF. The canonical pathway results in the release of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6, while the non-canonical pathway sees a release of Type I interferons into the microenvironment. (B) An established pathway for E2's modulatory effect on zebrafish toll-like receptor and estrogen receptor expression has not been entirely elucidated. Elevated estrogen levels in zebrafish were correlated with increased expression of both estrogen receptors and TLRs. Original Illustration by Michael S. Chembars.

Sun et al. postulates that heightened TLR expression results in overall greater NFxB expression within zebrafish hepatic and gonadal cells, due to the prevalence of estrogen receptors across multiple tissue types.³² However, the increased NFxB expression did not correlate with elevated inflammatory activity, but rather the opposite. In murine macrophages, it has been shown that E2 binding to its receptor EsrA can prevent p65 (also known as REIA) translocation through activation of the PI3K pathway.^{36, 37} The essential transcription factor p65 regulates NF-xB through its production of inhibitory factor IxB.³⁸ In another study, Valentine et al. determined that E2 can signal through both EsrA in addition to EsrB to suppress p65 in murine HeLa and COS-1 cells,³⁹ which further supports E2's ability to suppress NF-xB through the canonical pathway. Thus, in cell types that express the estrogen receptors, including important immune cells such as macrophages, E2 can function as a non-conventional anti-inflammatory drug and prevent the release of pro-inflammatory cytokines including TNF and IL-6 through inhibiting NF-xB.⁴⁰⁻⁴² In human models, E2-mediated depression is associated with post-menopausal osteoporosis during higher levels of secretion.^{35, 37, 42}

While E2 could potentially serve as a useful anti-inflammatory drug to treat several diseases in humans involving hyperimmune responses, its very inhibition of NF-xB and ultimately cytokine secretion could also cause adverse immunosuppressive effects as well. Previous studies with loss-of-function mutations that hinder or halt the TLR pathway have also been shown to cause immunodeficiencies, which in turn lead to recurrent bacterial infections, arthritis, and even cancer. ^{20, 43} The use of non-steroidal anti-inflammatory drugs (NSAIDs) to decrease estradiol levels in postmenopausal women is performed as an experimental technique for preventing breast cancer. ^{44, 45} However, estradiol's specific anti-inflammatory mechanisms and this correlation with carcinogenesis is not well understood, and requires further research. ⁴⁵ Additionally, research is available to suggest that zebrafish estrogen receptors may respond differently to environmental estrogens than human estrogen receptors. ⁴⁶

Thymus

The thymus is an important secondary immune organ, where T cells undergo much of their development, including V(d)J recombination and negative and positive selection.^{47, 48} There is also some evidence that estrogen exposure can cause aberrations in adaptive immune development as well. In teleost fish, research has identified that Esr2 is especially important in the ontogenesis of the thymus, and as such is the most probable of the ERs to bind to estrogen here.⁴⁹ A 'critical window' may exist during immune development, in relation to estrogen exposure, which may determine whether the hormone causes atrophy or hypertrophy of the thymus.^{50, 51} As a result, estrogen may have varying effects on this immune organ. There is not a lot of available data on the effects of natural estrogens such as E2 on thymic development in zebrafish, and thus further research is

needed to confirm the effect of natural estrogen exposure on thymic development in D. rerio. There is, however, research to support that synthetic estrogens such as EE2, or 17α -Ethnylestradiol, can cause thymic retardation in zebrafish embryos.⁵²

Notch & Hematopoiesis

Hematopoiesis is a highly regulated process that is responsible for the differentiation of self-renewing, multi-potent hematopoietic stem cells (HSCs) into lymphoid progenitor cells and myeloid progenitor cells.⁵³ The former is responsible for giving rise to T and B lymphocytes and other cells of adaptive immunity, while the latter is generally associated with the development of cells of the innate response, such as monocytes and granulocytes.⁵⁴ Before this differentiation occurs though, estrogen is shown to play an important role in zebrafish HSCs in the first 24-36 hours of development through Vegf/Notch signaling, through which it acts as a morphogen and signaling molecule.^{53,55} Carrol et al. additionally suggested that excess E2 exposure in this early phase led to a decrease in notch-family genes,⁵⁵ such as Notch3 and Notch1. Notch is a receptor that commits T-cell precursors towards the Tlymphocyte lineage by activating the GATA-3 transcription factor during the first double negative stage. Froehlicher et al. treated zebrafish embryos between 72 and 96 hours post fertilization (hpf) with a high concentration of an engineered ER\$2 morpholino (a molecule designed to modify gene expression). 56 By knocking out ERβ2 RNA translation with the morpholino, the exposure during embryonic development caused an upregulation of the Notch3 gene. ⁵⁶ In contrast, other studies which exposed mice to E2 demonstrated a correlation in which notch-family genes such as Notch1, and the Notch ligand Jagged1 were upregulated significantly, following higher concentrations of estradiol.⁵⁷⁻⁵⁹ The above differences between Carrol et al. versus other researchers who reported increased expression of notch-family genes could be due to one of several major experimental factors. Carrol et al. used a concentration of 8-10 µM estradiol chemical treatments in their study on embryos between 12 and 36 hpf.⁵⁵ In contrast, a treatment of 1 nM estradiol, in addition to using murine cell lines rather than zebrafish embryos, led Soares et al. to different results and conclusions.⁵⁷ Thus, while these differing results may be useful for studies concerning estrogen's effect on the environment, further research must be done to isolate which model organism (zebrafish or murine) is best for determining the effect of estrogen on hematopoiesis in *Homo sapiens*. Different avenues of research have also been proposed in zebrafish models to further solidify the understanding of estrogen's effect on Notch, including hippocampal formation and additional cell proliferation mechanisms.60

Overexpression of notch has been linked to highly increased canonical and non-canonical signaling through both the RBPJK signal binding protein and mTORC2 pathways, respectively.^{61,62} Additionally, Jagged1 overexpression may cause both positive and negative effects. In dystrophin deficient zebrafish with elevated levels of Jagged1, overexpression can rescue the mutant phenotypes in a particular model, opening up new avenues of research into treating Duchenne muscular dystrophy.⁶³ On the flipside, however, it is also linked to increased angiogenesis and cancer.⁶⁴ Particularly, when mTORC2 pathways were increased sharply, this resulted in decreased Foxp3 expression and activity. Foxp3 is a master gene regulator whose expression is sufficient to induce T lymphocyte differentiation; therefore, a decrease in Foxp3 will also result in a decrease in T lymphocyte expression. Mutations that decrease the expression or eliminate Notch altogether can also lead to decreased cardiac health and tissue regeneration in murine models.⁶⁵

Innate Cells

Innate immunity is often described as the first line of defense in the body's defense mechanism, and can include cell types such as eosinophils, basophils, neutrophils, and monocytes, the latter of which can differentiate into dendritic cells and macrophages.⁶⁶ Currently, it is thought that E2 primarily affects dendritic cell precursors and macrophages through Esra, although this is still under investigation.⁶⁷

Xu et al. reports that E2 exposure to whole male and female zebrafish embryos can also increase neutrophil counts by a factor of over 25%.68 This, along with elevated mpo and IL-8 gene expression, are indicative of elevated immune response stimulation. These results seem to be supported by several other studies as well. Namely, Rodriguez et al. used E2 in a zebrafish model to promote increased neutrophil expression and recruitment,69 along with TGFβ1, which resulted in metastasis of cancer cells. Because neutrophils and dendritic cells can derive from the same myeloid progenitor cell, one would also expect to see increased dendritic cell differentiation in response to E2 exposure. This is further demonstrated by Siracusa et al.'s murine study, which indeed found elevated MHC II+ dendritic cells after being exposed to E2 *in vivo*.70

Both neutrophils and dendritic cells are vital for innate immunity, as they are the most common immune cells which respond to infection and provide the greatest amount of antigen presentation, respectively, within the zebrafish immune arsenal. Neutrophils can phagocytose antigens, cause granule release, and release cytokines and chemokines that cause increased transmigration of additional white blood cells to the site of infection.²⁰ Dendritic cells, on the other hand, are the primary antigen presenting cells through their use of major histocompatibility complex (MHC) molecules and serve as the 'bridge' between the innate and adaptive branches of immunity.²⁰

There is some research available indicating that E2 can also affect macrophage migration to cancerous tissue via chemoattractants. E2 can increase circulating CCL2 and CCL5 chemokines, which are known tumorigenic compounds.^{71,72} The higher chemokine levels led to cancer metastasis due to the increased influx of macrophages.⁷¹ These results were similar to a zebrafish study by Rodriguez et al.,⁶⁹ which found that E2 can increase metastasis by inducing higher neutrophil recruitment and by promoting TGFβ1 expression. Estrogen also has regulatory effects on nitric oxide (NO) by increasing its release from macrophages.⁷³ Macrophages, which are well known for their production of inducible nitric oxide synthase (iNOS), utilize reactive oxygen and nitrogen species to kill captured organisms during phagocytosis.⁷⁴

Cytokines

Endocrine disrupting chemicals (EDCs) such as E2 have also been reported to have marked effects on expression of multiple cytokines within *Danio rerio*, such as TNFα, Type II interferons (IFN-γ), IL-1β, as well as the chemokines IL-8, CXCL-Clc, and CC-chemokine. The all found that TNFα, IL-1β, and IFN-γ expression levels were elevated in zebrafish embryos after exposure to higher concentrations of E2. In a different study, Xu et al. reported increased transcription of TNFα after exposure to E2,68 although their expression levels were lower when compared to those in Jin et al.'s experiment; In et al. exposed larvae to 12.5 μ g/L of E2, whereas Xu et al. treated larvae with only 5 μ g/L of E2, which could demonstrate a dose-dependent effect of E2 on TNFα. TNFα is the flagship cytokine of the TNF superfamily that is mainly released by macrophages, mast cells, and dendritic cells; its expression is regulated through the NF μ B pathway. Honor release, TNF μ C can cause a host of effects including apoptosis through proteins such as c-Jun, along with playing a major role in inducing inflammation, and activating MAP kinases. Note that the event of sepsis, activated macrophages might be apt to release large quantities of TNF μ C systemically, which can lead to septic shock due to a sharp drop in blood pressure. Lexcess amounts of TNF μ C have also been linked to arthritis and other inflammatory tissue diseases, such as vasculitis or ankylosing spondylitis. So, SI See Figure 2.

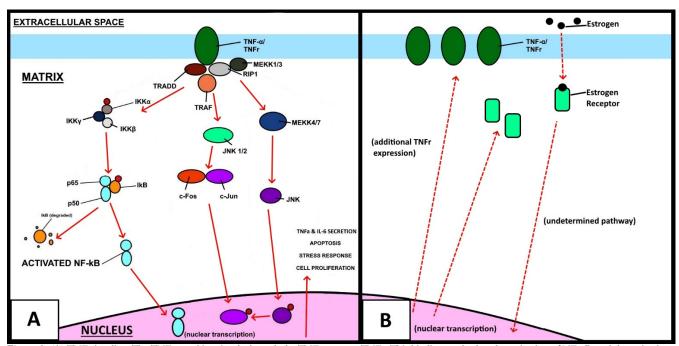


Figure 2. (A) TNF signaling. The TNF α cytokine signals through the TNF receptor, TNFr. This binding can lead to the activation of NF- α B, and the activation of cJun through the MEKK and JNK pathways. This can then lead to the release of chemicals that results in apoptosis, additional stress response, cell proliferation, and the release of additional pro-inflammatory cytokines. TNF α binding can cause a host of responses, but we chose to focus on those that are known to be affected by estrogen exposure. (B) An established pathway for E2's modulatory effect on TNF signaling has not been entirely elucidated. However, elevated estrogen levels in zebrafish were correlated with increased expression of both estrogen receptors and TNFr. Original Illustration by Michael S. Chembars.

IFN-γ is a type II interferon that is instrumental in modulating cell-mediated immunity, where it serves in multiple capacities including the amplification of antigen presentation, increasing reactive oxygen species (ROS) production for increased antiviral activity, and the induction of an anti-proliferative state to combat cancer.⁸² In the innate branch of immunity, ROS and IFN production is stimulated by macrophages, which secrete cytokines to attract natural killer cells to the area of inflammation and further IFN-γ production.^{83, 84} In a study by Xu et al., E2 exposure promoted higher expression of IFN-γ in both male and female zebrafish, ⁸⁵ which promoted the inflammatory response and led to increased cardiac tissue regeneration. Xu et al. also determined

that E2 caused Esr2a and Esr1 expression levels to increase in zebrafish, 85 while Esr2b expression was suppressed. Additional research also confirmed E2 binding to both Esr1 and GPER, which led to increased IFN-γ expression in zebrafish. 70, 86-88

E2 is also a known effector of the nitric oxide (NO) pathway. ⁸⁹ Jin et al. reinforced this by determining that E2 exposure can lead to an increase in iNOS gene expression, ⁷⁵ leading to its upregulation. Additional iNOS is expressed due to increased cytokine activity, and can cause the release of nitric oxide through catalysis. ^{75, 90, 91} Innate immune cells such as macrophages, neutrophils, or NK cells can utilize NO released to increase host resistance, lessen the severity of an infection, and serve as a potent antimicrobial and antiviral substance. ^{92, 93} Overabundant levels of iNOS in the body for a prolonged duration of time can lead to a plethora of autoimmune-induced diseases; these can include but are not limited to gastroenteritis, septic shock, or atherosclerosis. ⁹⁴ A different study by Karpuzoglu-Sahin et al. suggested similar overexpression of iNOS, ⁸⁷ this time due to its interaction with none other than the cytokine IFN-γ. ⁹⁵ This interferon's expression can be enhanced by E2 upstream as previously defined, which causes a cascade effect down the line with iNOS at the end of it, that eventually leads to an uncontrolled amount of NO released to the body. ⁷⁴

Current research also demonstrates that E2 exhibits a developmental stage-dependent effect on numerous genes, meaning the age at which the embryos are exposed to E2 in hours or days post fertilization (hpf or dpf, respectively) is crucial. One gene that regulates ubiquitination in zebrafish, *dcaf13* (human homolog: DCAF13), experienced a significant increase in expression at 1 dpf in zebrafish embryos following exposure to 1 µM E2.86 Ubiquitin is a protein which serves as the central player in both the processes of ubiquitination and its counterpart, deubiquitination; it is part of an immune retraction process that is essential for the regulation of both innate and adaptive responses.96 Ubiquitin accomplishes this through seven lysine residues, one of which can cause protein degradation through the 26s proteasome, while another named K63 can create linkages which are responsible for initiating cytokine signaling.97, 98 One of the 26s proteasome's functions is to degrade IxB, which subsequently frees NF-xB to enter the nucleus.99 Li et al. also expounded on ubiquitination's effect on the CbI family of proteins,97 which play a key role in the regulation of both T and B lymphocytes. Based on these reports, it is evident that E2 can affect other physiological processes through modulating ubiquitination, in addition to affecting zebrafish immune development.

The use and regulation of cytokines is crucial for a prompt and effective response from both the adaptive and innate repertoires of zebrafish immunity. Estrogen has demonstrated its ability to affect the expression and regulation of several cytokines through promoting gene transcription events. Further research should be conducted on other well-known cytokines and their related pathways in order to elucidate estrogen's specific method of action.

Micro-RNA Several studies have shown that E2 can influence microRNA regulation. MicroRNAs (miRNA) are small segments of RNA nucleotides that can silence gene expression post-transcriptionally by increasing the rate of degradation in mRNA.^{100, 101} In immunology, miRNAs have been identified to control multiple effects in immune cells, which affect all stages of development.¹⁰² Some miRNAs, such as miR-126 in zebrafish, have also been associated with regulating HSC differentiation, enabling them to control the myeloid and lymphoid lineages.¹⁰³ By comparison, in mice, miR-181 can increase B lymphocyte expression through lineage regulation.¹⁰⁴ A shift in lineage differentiation such as that caused by miR-181 may lead to the mutant organism exhibiting enhanced antibody production, at the expense of a heightened susceptibility to infection due to fewer myeloid innate cells that can professionally phagocytose.

E2 levels can either cause the upregulation of certain miRNA genes such as miR-17-92, or the downregulation of miRNA genes such as let7.^{105, 106} Several members of the miR-17-92 family were found to be upregulated in female zebrafish following exposure to E2.¹⁰⁵ miR-17-92 is responsible for regulating numerous cell types in the immune response, ranging from B lymphocyte development, T lymphocyte activation, and expression of dendritic cells.¹⁰⁷ In B lymphocytes, miR-17-92 expression is higher during the early stages of development, and decreases as the B lymphocytes reach the pre-B to immature B lymphocyte stages.¹⁰⁸ Overexpression or failure of miR-17-92 levels to decrease in T lymphocytes have been linked to several autoimmune diseases in both murine and human models, such as systemic lupus and multiple sclerosis.^{109, 110} One of the mechanisms through which it causes autoimmunity is through targeting several pro-apoptotic lipid phosphatases and proteins such as PTEN and Bim, respectively.^{107, 111} This in turn promotes Th1 delayed-type hypersensitivity, and simultaneously retarding regulatory T cell production.¹⁰⁷ Current knowledge on E2's role in zebrafish miR-17-92 expression is not extensively documented, and may present itself as an avenue of future research in lieu of less economical murine or human models.

A miRNA credited as a master regulator of inflammation, miR-155,¹¹² may play a role in promoting human breast cancer when exposure to E2 is involved.¹¹³ miR-155 is also present in zebrafish, though E2's effect in this organism is not well documented.¹¹⁴ Zebrafish models testing E2's effect on miR-155 could benefit current knowledge on human breast cancer, Gaucher's disease, and kidney disease.^{112, 114}

Cohen et al. discovered that E2 can also repress miRNA of the let-7 family in zebrafish; ¹⁰⁶ these are known tumor suppressants which regulate genes such as k-Ras. k-Ras is an oncogene which can signal through the PI3K and MAPK pathways, which eventually result in the execution of important cellular functions including cell proliferation; there are numerous studies that link k-Ras mutations in some form or fashion to approximately 25% of human cancers. ¹¹⁵ k-Ras can also cause secretion of proinflammatory cytokines such as IL-6, a cytokine noted for its tumorigenic activities. ^{115, 116} k-Ras can also mediate IL-1 and NF-×B expression, both of which are crucial to the inflammatory response. ^{115, 117} A study on k-Ras transgenic zebrafish has also suggested that estrogen plays some role in inhibiting cell proliferation in hepatocellular carcinoma (HCC). ^{118, 119} This discovery may help provide an answer as to why human males are more susceptible to HCC than females, as females traditionally express higher estrogen levels. ¹¹⁹

miRNA's suppression of k-RAS and other oncogenic genes continues to be studied in zebrafish, murine, and human models in order to fully determine the role of microRNA in regulating cancer. 120-122 As such, this is still an emerging field of study, and a promising one with many different avenues to pursue.

CONCLUSION

It is evident that estrogens, such as the naturally derived 17 β -estradiol, act as potent immunomodulatory agents in zebrafish. Many of these effects can alter critical pathways, such as the case of the complement cascade and the toxic buildup of Factor H, which reduces its effectiveness by preventing the formation of the membrane attack complex. Estrogen can affect pathways involving the expression of the protein NF- \varkappa B, which has effects ranging from development and maturation of immune cells to activation in response to infection. Estrogen can also upregulate cytokines such as TNF α , IL-1 β , and IFN- γ , which affect important functions such as apoptosis, mediating the inflammatory response, antigen presentation, and much more. Immunomodulatory effects can also be seen on microRNAs from estrogen exposure, which is a vital regulator in both myeloid and lymphoid lineage development.

EDCs are not exclusively natural estrogens such as E2. Other chemicals, such as bisphenols, phthalates, etc., can also signal through the estrogen receptor similarly to E2.^{123, 124} Because of an EDC's agonistic behavior, future research documenting their mechanisms of action to induce zebrafish immune disruption has been identified as a locale in research that should receive more attention, especially as it pertains to ecological toxicology.¹²⁵

These findings highlight the necessity for researchers to further investigate the effects of estrogenic compounds both in zebrafish and in other aquatic species, as these chemicals are becoming more prevalent in aquatic environments. ^{126, 127} From these environments, trace amounts of contaminants can radiate to terrestrial mammals including humans, primarily, though not exclusively, through the food supply. ¹²⁸⁻¹³⁰ Furthermore, estrogen's effect on many aspects of the immune system, especially the adaptive branch of immunity, is less understood. *Danio rerio* has proven itself as a staple model organism for immunological studies due to its cost efficiency, high embryo yield, and similarity to the human immune response. Through this model, it is suggested that additional research be conducted and documented concerning estrogen's effect on environmental and human health (as well as synthetic estrogens and estrogenic compounds such as bisphenol-A and its derivatives). ¹³¹

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REFERENCES

- Maruyama, Z., Yoshida, C.A., Furuichi, T., Amizuka, N., Ito, M., Fukuyama, R., Miyazaki, T., Kitaura, H., Nakamura, K., Fujita, T., Kanatani, N., Moriishi, T., Yamana, K., Liu, W., Kawaguchi, H., Nakamura, K., and Komori, T. (2007) Runx2 determines bone maturity and turnover rate in postnatal bone development and is involved in bone loss in estrogen deficiency, *Dev Dyn* 236, 1876-1890. https://doi.org/10.1002/dvdy.21187
- Zych, M., Kaczmarczyk-Sedlak, I., Wojnar, W., and Folwarczna, J. (2018) The Effects of Sinapic Acid on the Development of Metabolic Disorders Induced by Estrogen Deficiency in Rats, Oxid Med Cell Longev 2018. https://doi.org/10.1155/2018/9274246
- 3. Batavia, J.P.V., and Kolon, T.F. (2016) Fertility in disorders of sex development: A review, *J Pediatr Urol* 12, 418-425. https://doi.org/10.1016/j.jpurol.2016.09.015
- Simpson, E.R. (2003) Sources of estrogen and their importance, J Steroid Biochem Mol Biol 86, 225-230. https://doi.org/10.1016/S0960-0760(03)00360-1
- 5. Cutolo, M., Sulli, A., Capellino, S., Villaggio, B., Montagna, P., Seriolo, B., and Straub, R.H. (2004) Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity, *Lupus* 13, 635-638. https://doi.org/10.1191/0961203304lu1094oa

- 6. Menuet, A., Pellegrini, E., Anglade, I., Blaise, O., Laudet, V., Kah, O., and Pakdel, F. (2002) Molecular Characterization of Three Estrogen Receptor Forms in Zebrafish: Binding Characteristics, Transactivation Properties, and Tissue Distributions, Biol Reprod 66, 1881-1892. https://doi.org/10.1095/biolreprod66.6.1881
- Menuet, A., Page, Y.L., Torres, O., Kern, L., Kah, O., and Pakdel, F. (2004) Analysis of the estrogen regulation of the zebrafish estrogen receptor (ER) reveals distinct effects of ERalpha, ERbeta1 and ERbeta2, J Mol Endocrinol 32. https://doi.org/10.1677/jme.0.0320975
- Lopez-Munoz, A., Liarte, S., Gomez-Gonzalez, N.E., Cabas, I., Meseguer, J., Garcia-Ayala, A., and Mulero, V. (2015)
 Estrogen receptor 2b deficiency impairs the antiviral response of zebrafish, Dev Comp Immunol 53, 55-62.

 https://doi.org/10.1016/j.dci.2015.06.008
- Cabas. I., Rodenas, M.C., Abellan, E., Meseguer, J., Mulero, V., and Garcia-Ayala, A. (2013) Estrogen Signaling through the G Protein-Coupled Estrogen Receptor Regulates Granulocyte Activation in Fish, J Immunol 191, 4628-4639. https://doi.org/10.4049/jimmunol.1301613
- **10.** Burgos-Aceves, M.A., Cohen, A., Smith, Y., and Faggio, C. (2016) Estrogen regulation of gene expression in the teleost fish immune system, Fish Shellfish Immunol 58, 42-49. https://doi.org/10.1016/j.fsi.2016.09.006
- 11. Lassiter, C.S., Kelley, B., and Linney, E. (2002) Genomic structure and embryonic expression of estrogen receptor beta a (ERβa) in zebrafish (Danio rerio), *Gene* 299, 141-151. https://doi.org/10.1016/S0378-1119(02)01050-8
- 12. Bardet, P.L., Horard, B., Robinson-Rechavi, M., Laudet, V., and Vanacker, J.M., J Mol Endocrinol 28, 153-163. https://doi.org/10.1677/jme.0.0280153
- 13. Bhandari, R.K., Deem, S.L., Holliday, D.K., Jandegian, C.M., Kassotis, C.D., Nagel, S.C., Tillitt, D.E., vom Saal, F.S., and Rosenfeld, C.S. (2015) Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species, *Gen Comp Endocrinol* 214, 195-219. https://doi.org/10.1016/j.ygcen.2014.09.014
- **14.** Flint, S., Markle, T., Thompson, S., and Wallace, E. (2012) Bisphenol A exposure, effects, and policy: A wildlife perspective, *J Environ Manage* 104, 19-34. https://doi.org/10.1016/j.jenvman.2012.03.021
- **15.** Pinto, C., Hao, R., Grimaldi, M., Thrikawala, S., Boulahtouf, A., Ait-Aissa, S., Brion, F., Gustafsson, J.A., Balaguer, P., and Bondesson, M. (2019) Differential activity of BPA, BPAF and BPC on zebrafish estrogen receptors in vitro and in vivo, *Toxicol Appl Pharmacol* 380. https://doi.org/10.1016/j.taap.2019.114709
- **16.** Roy, J.R., Chakraborty, S., and Chakraborty, T.R. (2009) Estrogen-like endocrine disrupting chemicals affecting puberty in humans--a review, *Med Sci Monit* 15, 137-145.
- Qiao, Q., Liu, W., Wu, K., Song, T., Hu, J., Huang, X., Wen, J., Chen, L., and Zhang, X. (2013) Female zebrafish (Danio rerio) are more vulnerable than males to microcystin-LR exposure, without exhibiting estrogenic effects, *Aquat Toxicol* 142-143, 272-282. https://doi.org/10.1016/j.aquatox.2013.07.002
- **18.** Rose, J., Holbech, H., Lindholst, C., Norum, U., Povlsen, A., Korsgaard, B., and Bjerregaard, P. (2002) Vitellogenin induction by 17β-estradiol and 17α-ethinylestradiol in male zebrafish (Danio rerio), *Comp Biochem Physiol Part C: Toxicol Pharmacol*, 131, 531-539. https://doi.org/10.1016/S1532-0456(02)00035-2
- 19. Caspillo, N.R., Volkova, K., Hallgren, S., Olsson, P.E., and Porsch-Hallstrom, I. (2014) Short-term treatment of adult male zebrafish (Danio Rerio) with 17α-ethinyl estradiol affects the transcription of genes involved in development and male sex differentiation, Comp Biochem Physiol Part C: Toxicol Pharmacol, 164, 35-42. https://doi.org/10.1016/j.cbpc.2014.04.003
- 20. Murphy, K. (2012). Janeway's Immunobiology (Scobie, J., Ed.) 8th ed., Garland Science, New York.
- 21. Kumwenda, P., Cottier, F., Hendry, A.C., Gallagher, H., Tsai, H.J., and Hall, R.A. (2022) Estrogen promotes innate immune evasion of *Candida albicans* through inactivation of the alternative complement system, *Cell Rep* 38. https://doi.org/10.1016/j.celrep.2021.110183
- 22. Kopp, A., Hebecker, M., Svobodova, E., and Jozsi, M. (2012) Factor H: A Complement Regulator in Health and Disease, and a Mediator of Cellular Interactions, *Biomolecules* 2, 46-75. https://doi.org/10.3390/biom2010046
- 23. Prossnitz, E.R., and Barton, M. (2014) Estrogen Biology: New Insights into GPER Function and Clinical Opportunities, *Mol Cell Endocrinol* 389, 71-83. https://doi.org/10.1016/j.mce.2014.02.002
- 24. Hall, J.M., Couse, J.F., and Korach, K.S. (2001) The Multifaceted Mechanisms of Estradiol and Estrogen Receptor Signaling, *J Biol Chem* 276, 36869-36872. https://doi.org/10.1074/jbc.R100029200
- De-Cordoba, S.R., Gordillo-Esparza, J., De Jorge, E.G., Lopez-Trascasa, M., and Sanchez-Corral, P. (2004) The human complement factor H: functional roles, genetic variations and disease associations, *Mol Immunol* 41, 355-367. https://doi.org/10.1016/j.molimm.2004.02.005
- **26.** Ollert, MW., David, K., Bredehorst, R., and Vogel, C.W. (1995) Classical complement pathway activation on nucleated cells. Role of factor H in the control of deposited C3b, *J Immunol* 155, 4955-4962
- 27. Hogasen, K., Jansen, J.H., Mollnes, T.E., Hovdenes, J., and Harboe, M. (1995) Hereditary porcine membranoproliferative glomerulonephritis type II is caused by factor H deficiency, J Clin Investig 95, 1054-1061. https://doi.org/10.1172/JCI117751

- 28. Warwicker, P., Goodship, T.H.J., Donne, R.L., Pirson, Y., Nicholls, A., Ward, R.M., Turnpenny, P., and Goodship, J.A. (1998) Genetic studies into inherited and sporadic hemolytic uremic syndrome, *Kidney Int* 53, 836-844. https://doi.org/10.1111/j.1523-1755.1998.00824.x
- **29.** Miller, L.S., and Modlin, R.L. (2007) Toll-like receptors in the skin, *Semin Immunopathol* 29, 15-26. https://doi.org/10.1007/s00281-007-0061-8
- Kaisho, T., and Akira, S. (2006) Toll-like receptor function and signaling, J Allergy Clin Immunol 117, 979-987. https://doi.org/10.1016/j.jaci.2006.02.023
- **31.** Bekeredijian-Ding, I., and Jego, G. (2009) Toll-like receptors-sentries in the B-cell response, *Immunol* 128, 311-323. https://doi.org/10.1111/j.1365-2567.2009.03173.x
- 32. Sun, S.X., Zhang, Y.N., Lu, D.L., Wang, W.L., Limbu, S.M., Chen, L.Q., Zhang, M.L., and Du, Z.Y. (2019) Concentration-dependent effects of 17B-estradiol and bisphenol A on lipid deposition, inflammation and antioxidant response in male zebrafish (*Danio rerio*), Chemosphere 237, 124422. https://doi.org/10.1016/j.chemosphere.2019.124422
- 33. Mills, K.H.G. (2011) TLR-dependent T cell activation in autoimmunity, Nat Rev Immunol 11, 807-822. https://doi.org/10.1038/nri3095
- 34. Verstrepen, L., Bekaert, T., Chau, T.L., Tavernier, J., Chariot, A., and Beyaert, R. (2008) TLR-4, IL-1R and TNF-R signaling to NF-κB: variations on a common theme, *Cell Mol Life Sci* 65, 2964-2978. https://doi.org/10.1007/s00018-008-8064-8
- 35. Serasanambati, M., and Chilakapati, S.R. (2016) Function of Nuclear Factor kappa B (NF-νB) in human diseases-A Review, S Indian J Biol Sci 2, 368-387. https://doi.org/10.22205/sijbs/2016/v2/i4/103443
- 36. Ghisletti, S., Meda, C., Maggi, A., and Vegeto, E. (2005) 17B-Estradiol Inhibits Inflammatory Gene Expression by Controlling NF-xB Intracellular Localization, Mol Cell Biol 25, 2957-2968. https://doi.org/10.1128/MCB.25.8.2957-2968.2005
- **37.** Deshpande, R., Khalili, H., Pergolizzi, R.G., Michael, S.D., Chang, M.D.Y. (1997) Estradiol Down-Regulates LPS-Induced Cytokine Production and NFkB Activation in Murine Macrophages, *Am J Reprod Immunol* 38, 46-54. https://doi.org/10.1111/j.1600-0897.1997.tb00275.x
- 38. Scott, M.L., Fujita, T., Liou, H.C., Nolan, G.P., and Baltimore, D. (1993) The p65 subunit of NF-κB regulates IκB by two distinct mechanisms, *Genes Dev* 7, 1266-1276. https://doi.org/10.1101/gad.7.7a.1266
- **39.** Valentine, J.E., Kalkhoven, E., White, R., Hoare, S., and Parker, M.G. (2000) Mutations in the estrogen receptor ligand binding domain discriminate between hormone-dependent transactivation and transrepression, *J Biol Chem* 275, 25322-25329. https://doi.org/10.1074/jbc.M002497200
- **40.** Lee, S., Lee, M., Kim, J.B., Jo, A., Cho, E.J., Yu, S.J., Lee, J.H., Yoon, J.H., and Kim, Y.J. (2016) 17B-estradiol exerts anticancer effects in anoikis-resistant hepatocellular carcinoma cell lines by targeting IL-6/STAT3 signaling, *Biochem Biophys Res Commun* 473, 1247-1254. https://doi.org/10.1016/j.bbrc.2016.04.049
- 41. Mukherjee, U., Samanta, A., Biswas, S., Ghosh, S., Das, S., Banerjee, S., and Maitra, S. (2022) Chronic exposure to nonylphenol induces oxidative stress and liver damage in male zebrafish (*Danio rerio*): Mechanistic insight into cellular energy sensors, lipid accumulation and immune modulation. *Chem Biol Interact* 351, 109762. https://doi.org/10.1016/j.cbi.2021.109762
- **42.** Galien, R., and Garcia, T. (1997) Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site, *Nucleic Acids Res* **25**, 2424-2429. *https://doi.org/10.1093/nar/25.12.2424*
- **43.** Strickland, I., and Ghosh, S. (2006) Use of cell permeable NBD peptides for suppression of inflammation, *Ann Rheum Dis* 65, iii75-iii82. http://dx.doi.org/10.1136/ard.2006.058438
- 44. Byrns, M.C., and Penning, T.M. (2009) Type 5 17β-hydroxysteroid dehydrogenase/prostaglandin F synthase (AKR1C3): Role in breast cancer and inhibition by non-steroidal anti-inflammatory drug analogs, Chem Biol Interact 178, 1-3. https://doi.org/10.1016/j.cbi.2008.10.024
- **45.** Hudson, A.G., Gierach, G.L., Modugno, F., Simpson, J., Wilson, J.W., Evans, R.W., Vogel, V.G., and Weissfeld, J.L. (2008) Nonsteroidal Anti-inflammatory Drug Use and Serum Total Estradiol in Postmenopausal Women, *Cancer Epidemiol Biomarkers Prev* **17**, 680-687. https://doi.org/10.1158/1055-9965.EPI-07-2739
- **46.** Grimaldi, M., Boulahtouf, A., Delfosse, V., Thouennon, E., Bourguet, W., and Balaguer, P. (2015) Reporter cell lines to evaluate the selectivity of chemicals for human and zebrafish estrogen and peroxysome proliferator activated γ receptors, *Front Neurosci* 9. https://doi.org/10.3389/fnins.2015.00212
- **47.** Ma, D., Wei, Y., and Liu, F. (2013) Regulatory mechanisms of thymus and T cell development, *Dev Comp Immunol* 39, 91-102. https://doi.org/10.1016/j.dci.2011.12.013
- **48.** Takaba, H., and Takayanagi, H. (2017) The Mechanisms of T Cell Selection in the Thymus, *Trends Immunol* 38, 805-816. https://doi.org/10.1016/j.it.2017.07.010
- **49.** Moreira, C., Paiola, M., Duflot, A., Varo, I., Sitja-Bobadilla, A., Knigge, T., Pinto, P., and Monsinjon, T. (2021) The influence of 17B-oestradiol on lymphopoiesis and immune system ontogenesis in juvenile sea bass, *Dicentrarchus labrax*, *Dev Comp Immunol* 118, 104011. https://doi.org/10.1016/j.dci.2021.104011
- **50.** DeWitt, J.C., Peden-Adams, M.M., Keil, D.E., and Deitert, R.R. (2011) Current Status of Developmental Immunotoxicity: Early-Life Patterns and Testing, *Toxicol Pathol* 40, 230-236. https://doi.org/10.1177/0192623311427709

- 51. Paiola, M., Knigge, T., Picchietti, S., Duflot, A., Guerra, L., Pinto, P.I.S., Scapigliati, G., and Monsinjon, T. (2017) Oestrogen receptor distribution related to functional thymus anatomy of the European sea bass, *Dicentrarchus labrax*, *Dev Comp Immunol* 77, 106-120. https://doi.org/10.1016/j.dci.2017.07.023
- 52. Kernen, L., Phan, A., Bo, J., Herzog, E.L., Huynh, J., Segner, H., and Baumann, L. (2022) Estrogens as immunotoxicants: 17α-ethinylestradiol exposure retards thymus development in zebrafish (*Danio rerio*), Aquat Toxicol 242, 106025. https://doi.org/10.1016/j.aquatox.2021.106025
- 53. Nik, S., Weinreb, J.T., and Bowman, T.V. (2017) Developmental HSC Microenvironments: Lessons from Zebrafish, Stem Cell Microenviron Beyond 1041, 33-53. https://doi.org/10.1007/978-3-319-69194-7_4
- 54. Menke, A.L., Spitsbergen, J.M., Wolterbeek, A.P.M., and Woutersen, R.A. (2011) Normal Anatomy and Histology of the Adult Zebrafish, *Toxicol Pathol* 39, 759-775. https://doi.org/10.1177/0192623311409597
- 55. Carroll, K.J., Esain, V., Garnaas, M.K., Cortes, M., Dovey, M.C., Nissim, S., Frechette, G., Liu, S., Kwan, W., Cutting, C.C., Harris, J.M., Gorelick, D., Halpern, M., Lawson, N., Goessling, W., and North, T.E. (2014) Estrogen Defines the Dorsal-Ventral Limit of VEGF Regulation to Specify the Location of the Hemogenic Endothelial Niche, *Dev Cell* 29, 437-453. https://doi.org/10.1016/j.devcel.2014.04.012
- 56. Froehlicher, M., Liedtke, A., Groh, K., Lopez-Schier, H., Neuhass, S.C.F., Segner, H., and Eggen, R.I.L. (2009) Estrogen receptor subtype β2 is involved in neuromast development in zebrafish (*Danio rerio*) larvae, *Dev Biol* 330, 32-43. https://doi.org/10.1016/j.ydbio.2009.03.005
- 57. Soares, R., Balogh, G., Guo, S., Gartner, F., Russo, J., and Schmitt, F. (2004) Evidence for the Notch Signaling Pathway on the Role of Estrogen in Angiogenesis, *Mol Endocrinol* 18, 2333-2343. https://doi.org/10.1210/me.2003-0362
- 58. Fan, J.Z., Yang, L., Meng, G.L., Lin, Y.S., Wei, B.Y., Fan, J., Hu, H.M., Liu, Y.W., Chen, S., Zhang, J.K., He, Q.Z., Luo, Z.J., and Liu, J. (2014) Estrogen improves the proliferation and differentiation of hBMSCs derived from postmenopausal osteoporosis through notch signaling pathway, *Mol Cell Biochem* 392, 85-93. https://doi.org/10.1007/s11010-014-2021-7
- Radtke, F., Wilson, A., and MacDonald, H.R. (2004) Notch signaling in T- and B-cell development, Current Opinion in Immunology 16, 174-179. https://doi.org/10.1016/j.coi.2004.01.002
- 60. Diotel, N., Vaillant, C., Gabbero, C., Mironov, S., Fostier, A., Gueguen, M.M., Anglade, I., Kah, O., and Pellegrini, E. (2013) Effects of estradiol in adult neurogenesis and brain repair in zebrafish, *Horm Behav* 63, 193-207. https://doi.org/10.1016/j.yhbeh.2012.04.003
- **61.** Kasper, I.R., Apostolidis, S.A., Sharabi, A., and Tsokos, G.C. (2016) Empowering Regulatory T Cells in Autoimmunity, *Trends Mol Med* 22, 784-797. https://doi.org/10.1016/j.molmed.2016.07.003
- **62.** Charbonnier, L.M., Wang, S., Georgiev, P., Sefik, E., and Chatila, T.A. (2015) Control of peripheral tolerance by regulatory T cell-intrinsic Notch signaling, *Nat Immunol* 16, 1162-1173. https://doi.org/10.1038/ni.3288
- 63. Vieira, N.M., Elvers, I., Alexander, M.S., Moreira, Y.B., Eran, A., Gomes, J.P., Marshall, J.L., Karlsson, E.K., Verjovski-Almeida, S., Lindblad-Toh, K., Kunkel, L.M., and Zatz, M. (2015) Jagged 1 Rescues the Duchenne Muscular Dystrophy Phenotype, *Cell* 163, 1204-1213. https://doi.org/10.1016/j.cell.2015.10.049
- 64. Benedito, R., Roca, C., Sorensen, I., Adams, S., Gossler, A., Fruttiger, M., and Adams, R.H. (2009) The Notch Ligands Dll4 and Jagged1 Have Opposing Effects on Angiogenesis, Cell 137, 1124-1135. https://doi.org/10.1016/j.cell.2009.03.025
- 65. Li, Y., Hiroi, Y., and Liao, J.K. (2010) Notch Signaling as an Important Mediator of Cardiac Repair and Regeneration After Myocardial Infarction, *Trends Cardiovasc Med* 20, 228-231. https://doi.org/10.1016/j.tcm.2011.11.006
- **66.** Xu, H., Yang, M., Qiu, W., Pan, C., and Wu, M. (2013) The impact of endocrine-disrupting chemicals on oxidative stress and innate immune response in zebrafish embryos, *Environ Toxicol* 32, 1793-1799. https://doi.org/10.1002/etc.2245
- **67.** Kovats, S. (2015) Estrogen receptors regulate innate immune cells and signaling pathways, *Cell Immunol* 294, 63-69. https://doi.org/10.1016/j.cellimm.2015.01.018
- 68. Xu, H., Zhang, X., Li, H., Li, C., Huo, X.J., Hou, L.P., and Gong, Z. (2018) Immune response induced by major environmental pollutants through altering neutrophils in zebrafish larvae. *Aquat Toxicol* 201, 99-108. https://doi.org/10.1016/j.aquatox.2018.06.002
- Rodriguez, G.V., Abrahamsson, A., Jensen, L.D.E., and Dabrosin, C. (2017) Estradiol Promotes Breast Cancer Cell Migration via Recruitment and Activation of Neutrophils, Cancer Immunol Res 5, 234-247. https://doi.org/10.1158/2326-6066.CIR-16-0150
- Siracusa, M.C., Overstreet, M.G., Housseau, F., Scott, A.L., and Klein, S.L. (2007) 17B-Estradiol Alters the Activity of Conventional and IFN-Producing Killer Dendritic Cells, *J Immunol* 180, 1423-1431. https://doi.org/10.4049/jimmunol.180.3.1423
- Svennson, S., Abrahamsson, A., Rodriguez, G.V., Olsson, A.K., Jensen, L., Cao, Y., and Dabrosin, C. (2015) CCL2 and CCL5 are Novel Therapeutic Targets for Estrogen-Dependent Breast Cancer, Clin Cancer Res 21, 3794-3805. https://doi.org/10.1158/1078-0432.CCR-15-0204
- 72. Soria, G., and Ben-Baruch-A. (2008) The inflammatory chemokines CCL2 and CCL5 in breast cancer, Cancer Lett 267, 271-285. https://doi.org/10.1016/j.canlet.2008.03.018

- 73. You, H.J., Kim, J.Y., and Jeong, H.G. (2003) 17β-Estradiol increases inducible nitric oxide synthase expression in macrophages, *Biochem Biophys Res Commun* 303, 1129-1134. https://doi.org/10.1016/S0006-291X(03)00477-7
- 74. Karpuzoglu, E., and Ahmed, S.A. (2006) Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: Implications for immunity, autoimmune diseases, and apoptosis, *Nitric Oxide* 15, 177-186. https://doi.org/10.1016/j.niox.2006.03.009
- 75. Jin, Y., Chen, R., Liu, W., and Fu, Z. (2010) Effect of endocrine disrupting chemicals on the transcription of genes related to the innate immune system in the early developmental stage of zebrafish (*Danio rerio*), Fish Shellfish Immunol 28, 854-861. https://doi.org/10.1016/j.fsi.2010.02.009
- **76.** Cavaillon, J.M. (1994) Cytokines and macrophages, *Biomed Pharmacother* 48, 445-453. https://doi.org/10.1016/0753-3322(94)90005-1
- 77. Ye, C., Bhan, A.K., Deshpande, V., Shankar, P., and Manjunath, N. (2013) Silencing TNF-α in macrophages and dendritic cells for arthritis treatment, Scand J Rheumatol 42, 266-269. https://doi.org/10.3109/03009742.2013.777779
- 78. Larrick, J.W., and Wright, S.C. (1990) Cytotoxic mechanism of tumor necrosis factor-a, J Fed Am Soc Exp Biol 4, 3215-3223. https://doi.org/10.1096/fasebj.4.14.2172061
- 79. Ebmeyer, J., Leichtle, A., Hernandez, M., Ebmeyer, U., Husseman, J., Pak, K., Sudhoff, H., Broide, D., Wasserman, S.I., and Ryan, A.F. (2011) TNFA deletion alters apoptosis as well as caspase 3 and 4 expression during otitis media, *BMC Immunol* 12. https://doi.org/10.1186/1471-2172-12
- **80.** Sabio, G., and Davis, R.J. (2014) TNF and MAP kinase signaling pathways, *Semin Immunol* 26, 237-245. https://doi.org/10.1016/j.smim.2014.02.009
- 81. Wong, M., Ziring, D., Korin, Y., Desai, S., Kim, S., Lin, J., Gjertson, D., Braun, J., Reed, E., and Singh, R.R. (2008) TNFα blockade in human diseases: Mechanisms and future directions, *J Clin Immunol* 126, 121-136. https://doi.org/10.1016/j.clim.2007.08.013
- 82. Kak, G., Mohsin, R., and Tiwari, B.K. (2018) Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases, Biomol Concepts 9, 64-79. https://doi.org/10.1515/bmc-2018-0007
- 83. Gessani, S., and Belardelli, F. (1998) IFN-γ Expression in Macrophages and Its Possible Biological Significance, *Cytokine Growth Factor Rev* 9, 117-123. https://doi.org/10.1016/S1359-6101(98)00007-0
- **84.** Salazar-Mather, T.P., Hamilton, T.A., and Biron, C.A. (2000) A chemokine-to-cytokine-to-chemokine cascade critical in antiviral defense, *J Clin Investig* 105, 985-993. https://doi.org/10.1172/JCI9232
- 85. Xu, S., Xie, F., Tian, L., Fallah, S., Babaei, F., Manno, S.H.C., Manno III, F.A.M., Zhu, L., Wong, K.F., Liang, Y., Ramalingam, R., Sun, L., Wang, X., Plumb, R., Gethings, L., Lam, Y.W., and Cheng, S.H. (2020) Estrogen accelerates heart regeneration by promoting the inflammatory response in zebrafish, *J Endocrinol* 245, 39-51. https://doi.org/10.1530/JOE-19-0413
- **86.** Hao, R., Bondesson, M., Singh, A.V., Riu, A., McCollum, C.W., Knudsen, T.B., Gorelick, D.A., and Gustafsson, J.A. (2013) Identification of Estrogen Target Genes during Zebrafish Embryonic Development through Transcriptomic Analysis, *PLoS ONE 8. https://doi.org/10.1371/journal.pone.0079020*
- 87. Karpuzoglu-Sahin, E., Hissong, B.D., and Ahmed, S.A. (2001) Interferon-y levels are upregulated by 17-B-estradiol and diethylstilbestrol, *J Reprod Immunol* 52, 113-127. https://doi.org/10.1016/S0165-0378(01)00117-6
- 88. Dai, R., Philips, R.A., Zhang, Y., Khan, D., Crasta, D., and Ahmed, S.A. (2008) Suppression of LPS-induced Interferon-y and nitric oxide in splenic lymphocytes by select estrogen-regulated microRNAs: a novel mechanism of immune modulation, *Am Soc Hematol* 112, 4591-4597. https://doi.org/10.1182/blood-2008-04-152488
- 89. Sykes, B.G., Van Steyn, P.M., Vignali, J.D., Winalski, J., Lozier, J., Bell, W.E., and Turner, J.E. (2016) The Relationship between Estrogen and Nitric Oxide in the Prevention of Cardiac and Vascular Anomalies in the Developing Zebrafish (*Danio rerio*), *Brain Sci* 6, 51. https://doi.org/10.3390/brainsci6040051
- **90.** Forstermann, U., and Sessa, W.C. (2012) Nitric oxide synthases: regulation and function, *Eur Heart J* 33, 829-837. https://doi.org/10.1093/eurheartj/ehr304
- **91.** Taylor, B.S., and Geller, D.A. (2000) Molecular Regulation of the Human Inducible Nitric Oxide Synthase (iNOS) Gene, *Shock* 13, 413-424. https://doi.org/10.1097/00024382-200006000-00001
- 92. Tripathi, P. (2007) Nitric oxide and immune response, Indian J Biochem Biophys 44, 310-319.
- 93. MacMicking, J., Qiao-wen, X., and Nathan, C. (1997) Nitric oxide and macrophage function, *Annu Rev Immunol* 15, 323-350. https://doi.org/10.1146/annurev.immunol.15.1.323
- 94. Kleinert, H., and Forstermann, U. (2007) Inducible Nitric Oxide Synthase, in XPharm: The Comprehensive Pharmacology Reference (Enna, S.J., and Bylund, D.B., Ed.) 1-12, Elsevier. https://doi.org/10.1016/B978-008055232-3.60509-4
- 95. Bogdan, C. (2000) The Function of Nitric Oxide in the Immune System, in *Handbook of Experimental Pharmacology*. (Mayer, B., Ed.) 443–492, Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-57077-3_19
- **96.** Zinngrebe, J., Montinaro, A., Peltzer, N., and Walczak, H. (2014) Ubiquitin in the immune system, Eur Mol Biol Organ Rep 15, 28-45. https://doi.org/10.1002/embr.201338025

- **97.** Li, X., Gong, L., and Gu, H. (2019) Regulation of immune system development and function by CbI-mediated ubiquitination, *Immunol Rev* 291, 123-133. https://doi.org/10.1111/imr.12789
- Chen, Z.J., and Sun, L.J. (2009) Nonproteolytic Functions of Ubiquitin in Cell Signaling, Mol Cell 33, 275-286. https://doi.org/10.1016/j.molcel.2009.01.014
- 99. Chen, Z.J. (2005) Ubiquitin signaling in the NF-xB pathway, Nat Cell Biol 7, 758-765. https://doi.org/10.1038/ncb0805-758
- **100.** Ha, M., and Kim, V.N. (2014) Regulation of mciroRNA biogenesis, *Nat Rev Mol Cell Biol* 15, 509-524. https://doi.org/10.1038/nrm3838
- **101.** Jonas, S., and Izaurralde, E. (2015) Towards a molecular understanding of microRNA-mediated gene silencing, *Nat Rev Genet* 16, 421-433. https://doi.org/10.1038/nrg3965
- **102.** Lu, T.X., and Rothenberg, M.E. (2017) MicroRNA, J Allergy Clin Immunol 141, 1202-1207. https://doi.org/10.1016/j.jaci.2017.08.034
- 103. Grabher, C., Payne, E.M., Johnston, A.B., Bolli, N., Lechman, E., Dick, J.E., Kanki, J.P., and Look, A.T. (2011) Zebrafish microRNA-126 determines hematopoietic cell fate through c-Myb, *Leukemia* 25, 506-514. https://doi.org/10.1038/leu.2010.280
- 104. Chen, C.Z., Li, L., Lodish, H.F., and Bartel, D.P. (2004) MicroRNAs Modulate Hematopoietic Lineage Differentiation, Science 303, 83-86. https://doi.org/10.1126/science.1091903
- 105. Cohen, A., and Smith, Y. (2014) Estrogen Regulation of microRNAs, Target Genes, and microRNA Expression Associated with Vitellogenesis in the Zebrafish, Zebrafish 11, 462-478. https://doi.org/10.1089/zeb.2013.0873
- 106. Cohen, A., Burgos-Aceves, M.A., Kahan, T., and Smith, Y. (2017) Estrogen Repression of MicroRNAs is Associated with High Guanine Content in the Terminal Loop Sequences of Their Precursors, Biomedicines 5, 47. https://doi.org/10.3390/biomedicines5030047
- **107.** Kuo, G., Wu, C.Y., and Yang, H.Y. (2019) MiR-17-92 cluster and immunity, *J Formos Med Assoc* 118, 2-6. https://doi.org/10.1016/j.jfma.2018.04.013
- 108. Spierings, D.C., McGoldrick, D., Hamilton-Easton, A.M., Neale, G., Murchison, E.P., Hannon, G.J., Green, D.R., and Withoff, S. (2011) Ordered progression of stage-specific miRNA profiles in the mouse B2 B-cell lineage, *Blood* 117, 5340-5349. https://doi.org/10.1182/blood-2010-10-316034
- 109. Lindberg, R.L.P., Hoffman, F., Mehling, M., Kuhle, J., and Kappos, L. (2010) Altered expression of miR-17-5p in CD4+lymphocytes of relapsing-remitting multiple sclerosis patients, Eur J Immunol 40, 888-898.
 https://doi.org/10.1002/eji.200940032
- 110. Dai, R., Zhang, Y., Khan, D., Heid, B., Caudell, D., Crasta, O., and Ahmed, S.A. (2010) Identification of a Common Lupus Disease-Associated microRNA Expression Pattern in Three Different Murine Models of Lupus, PLoS ONE 5. https://doi.org/10.1371/journal.pone.0014302
- 111. Yamada, K.M., and Araki, M. (2001) Tumor suppressor PTEN: modulator of cell signaling, growth, migration, and apoptosis, J Cell Sci 114, 2375-2382. https://doi.org/10.1242/jcs.114.13.2375
- 112. Watson, L., Keatinge, M., Gegg, M., Bai, Q., Sandulescu, M.C., Vardi, A., Futerman, A.H., Schapira, A.H.V., Burton, E.A., and Bandmann, O. (2019) Ablation of the pro-inflammatory master regulator miR-155 does not mitigate neuroinflammation or neurodegeneration in a vertebrate model of Gaucher's disease, Neurobiol Dis 127, 563-569. https://doi.org/10.1016/j.nbd.2019.04.008
- 113. Zhang, C., Zhao, J., and Deng, H. (2013) 17β-Estradiol up-regulates miR-155 expression and reduces TP53INP1 expression in MCF-7 breast cancer cells, Mol Cell Biochem 379, 201-211. https://doi.org/10.1007/s11010-013-1642-6
- **114.** Morales, E.E., and Wingert, R.A. (2017) Zebrafish as a Model of Kidney Disease, *Kidney Dev Dis* 60, 55-75. https://doi.org/10.1007/978-3-319-51436-9_3
- 115. Hamarsheh, S., Grob, O., Brummer, T., and Zeiser, R. (2020) Immune modulatory effects of oncogenic KRAS in cancer, Nat Commun 11. https://doi.org/10.1038/s41467-020-19288-6
- 116. Miller, A., McLeod, L., Alhayyani, S., Szczepny, A., Watkins, D.N., Chen, W., Enriori, P., Ferlin, W., Ruwanpura, S., and Jenkins, B.J. (2017) Blockade of the IL-6 trans-signalling/STAT3 axis suppresses cachexia in Kras-induced lung adenocarcinoma, Oncogene 36, 3059-3066. https://doi.org/10.1038/onc.2016.437
- 117. Dinarello, C.A. (1997) Interleukin-1, Cytokine Growth Factor Rev 8, 253-265. https://doi.org/10.1016/s1359-6101(97)00023-3
- **118.** Li, Y., Li, H., and Gong, Z. (2017) Males develop faster and more severe hepatocellular carcinoma than females in krasV12 transgenic zebrafish, *Sci Rep 7. https://doi.org/10.1038/srep41280*
- 119. Li, H., Lu, J.W., Huo, X., Li, Y., Li, Z., and Gong, Z. (2019) Effects of sex hormones on liver tumor progression and regression in Myc/xmrk double oncogene transgenic zebrafish, Gen Comp Endocrinol 277, 112-121. https://doi.org/10.1016/j.ygcen.2019.03.018
- **120.** Lai, C.Y., Yeh, K.Y., Liu, B.F., Chang, T.M., Chang, C.H., Liao, Y.F., Liu, Y.W., and Her, G.M. (2021) MicroRNA-21 Plays Multiple Oncometabolic Roles in Colitis-Associated Carcinoma and Colorectal Cancer via the PI3K/AKT, STAT3, and PDCD4/TNF-α Signaling Pathways in Zebrafish, *Cancers* 13. https://doi.org/10.3390/cancers13215565
- **121.** Shui, B., La Rocca, G., Ventura, A., and Haigis, K.M. (2022) Interplay between K-RAS and miRNAs, *Trends Cancer* 8, 384-396. https://doi.org/10.1016/j.trecan.2022.01.002

- **122.** Lundberg, I.V., Wikberg, M.L., Ljuslinder, I., Li, X., Myte, R., Zingmark, C., Lofgren-Burstrom, A., Edin, S., and Palmqvist, R. (2018) MicroRNA Expression in KRAS- and BRAF-mutated Colorectal Cancers, Anticancer Res 38, 677-683.
- 123. Moreman, J., Lee, O., Trznadel, M., David, A., Kudoh, T., and Tyler, C.R. (2017) Acute Toxicity, Teratogenic, and Estrogenic Effects of Bisphenol A and Its Alternative Replacements Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish Embryo-Larvae, Environ Sci Technol 51, 12796-12805. https://doi.org/10.1021/acs.est.7b03283
- 124. Lee, H., Lee, J., Choi, K., and Kim, K.T. (2019) Comparative analysis of endocrine disrupting effects of major phthalates in employed two cell lines (MVLN and H295R) and embryonic zebrafish assay, Environ Res 172, 319-325. https://doi.org/10.1016/j.envres.2019.02.033
- 125. Huang, Y., Wang, X.L., Zhang, J.W., and Wu, K.S. (2014) Impact of Endocrine-Disrupting Chemicals on Reproductive Function in Zebrafish (*Danio rerio*), Reprod Domest Anim 50, 1-6. https://doi.org/10.1111/rda.12468
- 126. Fang, Z., Gao, Y., Wu, X., Xu, X., Sarmah, A.K., Bolan, N., Gao, B., Shaheen, S.M., Rinklebe, J., Ok, Y.S., and Wang, H. (2019) A critical review on remediation of bisphenol S (BPS) contaminated water: Efficacy and mechanisms, *Crit Rev Environ Sci Technol* 50, 476-522. https://doi.org/10.1080/10643389.2019.1629802
- 127. Catenza, C.J., Farooq, A., Shubear, N.S., and Donkor, K.K. (2021) A targeted review n fate, occurrence, risk and health implications of bisphenol analogues, *Chemosphere* 268. https://doi.org/10.1016/j.chemosphere.2020.129273
- **128.** Mercogliano, R., and Santonicola, S. (2018) Investigation on bisphenol A levels in human milk and dairy supply chain: A review, Food Chem Toxicol 114, 98-107. https://doi.org/10.1016/j.fct.2018.02.021
- **129.** Olsvik, P.A., Lie, K.K., Sturve, J., Hasselberg, L., and Andersen, O.K. (2009) Transcriptional effects of nonylphenol, bisphenol A and PBDE-47 in liver of juvenile Atlantic cod (*Gadus morhua*), *Chemosphere* 75, 360-367. https://doi.org/10.1016/j.chemosphere.2008.12.039
- **130.** Olsvik, P.A., Skjaerven, K.H., and Softeland, L. (2017) Metabolic signatures of bisphenol A and genistein in Atlantic salmon liver cells, *Chemosphere* 189, 730-743. https://doi.org/10.1016/j.chemosphere.2017.09.076
- 131. Meeker, N.D., and Trede, N.S. (2008) Immunology and zebrafish: Spawning new models of human disease, *Dev Comp Immunol* 32, 745-757. https://doi.org/10.1016/j.dci.2007.11.011

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PRESS SUMMARY

This study reviews current knowledge of the effect of estrogen on immune system efficacy in zebrafish. Significant research has been conducted on this topic, and primarily concludes that estrogen exposure can cause aberrant immunological development and function that decreases the overall health of the organism.

