CANADIAN JOURNAL of UNDER GRADUATE RESEARCH

Epigenetic Regulation of Fatty Liver Disease

"…in addition to enhancing disease progression, these histone modifications can conversely act to protect from harmful side effects of this disease." (p.8)

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Analysis of Parliamentary Debates on the Canadian Housing Crisis

"The research identifies six dominant frames, with excessivefederal spending and excessive bureaucracy emerging as the most prominent." (p. 13)

Trauma-Informed Approaches in Prisons for Federally Sentenced Women

"This paper offers policy change recommendations, including eliminating strip searches and providing correctional programming led by external treatment providers, that would minimize the harm women experience while incarcerated" (p. 18)

CANADIAN JOURNAL *of* UNDERGRADUATE RESEARCH

A student-led publication that aims to highlight research by undergraduate students of all disciplines

Second Issue OF THE Ninth Volume
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This issue is published on the traditional, ancestral, and unceded territory of the Coast Salish Nations, including xʷməθkʷəy̓əm (Musqueam), Sḵwx̱wú7mesh (Squamish), and səlilwətaɬ (Tsleil-Waututh).

Letter from the editors-in-chief


We are thrilled to present Volume 9 Issue 2 of the Canadian Journal of Undergraduate Research (CJUR). This issue features seven articles from undergraduate students across Canada, with research topics as diverse as protein structures, the Canadian housing crisis, trauma-informed approaches in prisons for federally sentenced women, pharmacy students' readiness to prescribe in British Columbia, lake primary producer community responses to anthropogenic use, microglia-specific genetic factors in autism spectrum disorders, and effects of carbon dioxide fertilization and copper exposure on photosynthesis. The CJUR team is excited and honoured to be able to provide a platform for showcasing the outstanding work of our Canadian undergraduate contributors.

This year, we have received 36 new manuscripts from undergraduate authors, all of which were of exceptional quality and from various disciplines. We were continually impressed by the exceptional work that we received throughout the year, and are beyond grateful to our authors for trusting us with the outcome of their scholarly endeavors. We are also sincerely appreciative of our graduate, postdoctoral, and faculty reviewers, without whose support this edition could not have been accomplished.

As our academic year comes to an end, we would also like to acknowledge and recognize the outstanding efforts demonstrated by our editorial team. Our editors have worked relentlessly to ensure efficient communication with authors and reviewers while managing full-time jobs, classes, and other commitments. It was because of our team that we were able to accept many manuscripts, establish collaborations, promote CJUR, and provide publishing opportunities to as many Canadian undergraduate researchers as possible. We are also thankful of our senior advisors and core team at UBC Undergraduate Research Opportunities (URO) for offering us their unconditional support.

It is our hope that you will enjoy Volume 9 Issue 2 as much as we did, and we thank you for your continued support of CJUR.

Yours truly,

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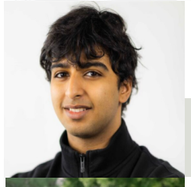

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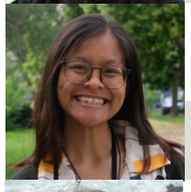

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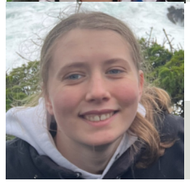

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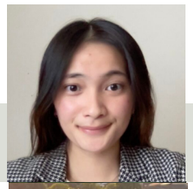

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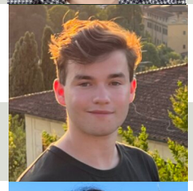

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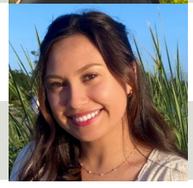

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The role of histone demethylases in the epigenetic regulation of non-alcoholic fatty liver disease

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ABSTRACT An ever-increasing rise in the occurrence of non-alcoholic fatty liver disease (NAFLD) presents a need to understand its development and progression with a goal of advancing novel treatment options. Epigenetics encompasses numerous modifications that mediate gene regulation amidst diverse genetic and environmental conditions. Specifically, histone methylations play a key role in the regulation of lipogenic gene expression and, like other epigenetic modifications, are inherently reversible. This presents an array of options for therapeutic intervention. This review explores the roles of specific histone demethylases and their dynamic relationships with transcription factors and target genes involved in NAFLD. I summarize how, in addition to enhancing disease progression, these histone modifications can conversely act to protect from harmful side effects of this disease. I suggest areas of future investigation into additional transcription and epigenetic factors that have yet to be studied in this context and are necessary to further our understanding of NAFLD and to develop novel treatment options.

INTRODUCTION

In a time of fast food giants and prolific delivery services, a life reliant on a high fat diet and lack of exercise is easier than ever to achieve, but it comes at a cost to our health. The rise in obesity and type 2 diabetes is paralleled by a rise in the leading cause of chronic liver disease: non-alcoholic fatty liver disease (NAFLD) (Le et al., 2022). NAFLD is characterized by greater than 5% fat accumulation in hepatocytes without excessive alcohol use, and at present, affects 25-30% of the global population (Le et al., 2022). The occurrence of NAFLD is not restricted to obese individuals, and as such, its prevalence is projected to outpace a global rise in obesity and reach 55.4% by 2040 (Le et al., 2022). Understanding this development and progression of this disease is essential to advance novel treatment options.

The multifactorial progression of NAFLD through non-alcoholic steatohepatitis (NASH), hepatic cirrhosis, and, in some cases, hepatocellular carcinoma, is presented in Figure 1 (Juanola et al., 2021). Genome-wide association studies have identified numerous single nucleotide polymorphisms in hepatic lipid regulation genes (Juanola et al., 2021). Environmental factors and demographics such as a lack of exercise, poor diet, and smoking have been linked to NAFLD development to varying degrees (Juanola et al., 2021). Primarily, obesity and its characteristic increase in *de novo* lipogenesis (DNL) increases the accumulation of fatty acids that can lead to insulin resistance, type 2 diabetes, and cardiovascular disease which all participate in the development of NAFLD (Sodum et al., 2021). Lifestyle changes including diet and exercise are the key preventative treatment options for NAFLD and currently are the only available options for reversing disease progression. A loss of ~10% body weight has been equated to an improvement in NASH in 90% of patients and fibrosis regression in ~45% of patients (Nassir, 2022). However, reductions in steatosis and fibrosis have been observed with a loss of body weight as low as 3-5% (Nassir, 2022). Furthermore, symptom management is typically achieved through insulin sensitizers, lipid lowering agents, antioxidants, and anti-inflammatory agents, among others (Sodum et al., 2021).

In order to fit in the nucleus, DNA is wound around histone proteins to form nucleosomes that are progressively condensed into chromatin (Peterson & Laniel, 2004). Each histone has a 20-35 amino acid N-terminal tail that protrudes out from the histone octamer (Peterson & Laniel, 2004). These N-terminal tails interact with adjacent nucleosomes and are sites for post-transcriptional modification by enzymes, such as histone demethylases, to alter chromatin structure and gene expression (Figure 2). Generally, epigenetic modifications are inherently reversible within very short time periods, which presents a vast area of therapeutic

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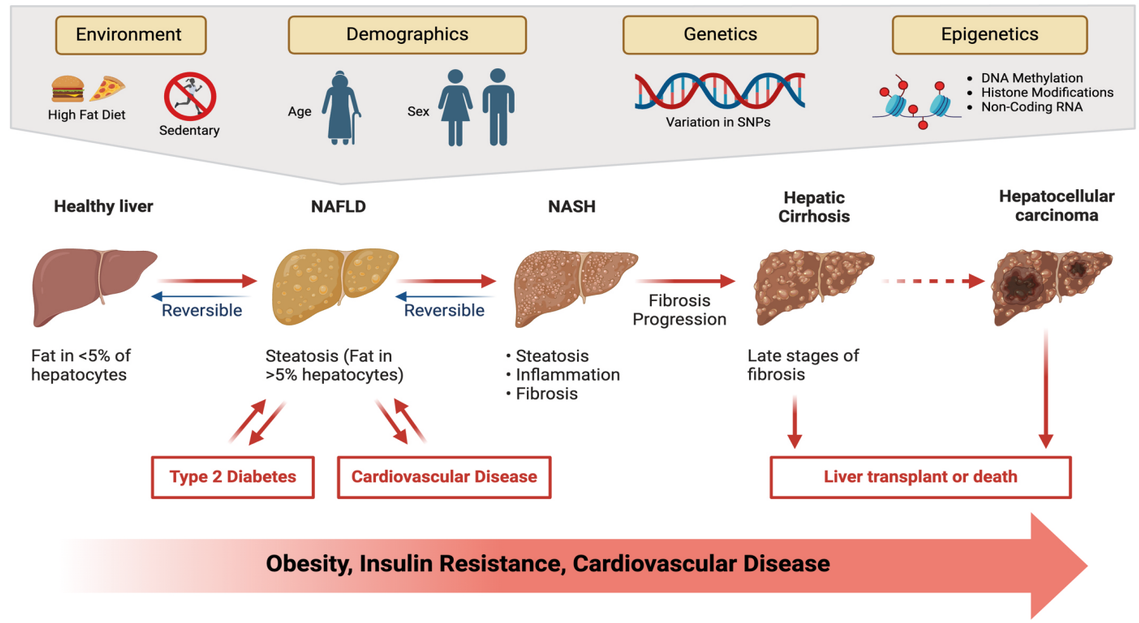

Figure 1 *The multifactorial influences and subsequent progression of non-alcoholic fatty liver disease.* Environmental, demographic, genetic, and epigenetic factors lead to an accumulation of fat in hepatocytes. The resulting onset of NAFLD is followed by the progression into hepatic cirrhosis and hepatocellular carcinoma characterized by inflammation and fibrosis development (Buzzetti et al., 2016, Juanola et al., 2021). Image adapted from "Non-Alcoholic Fatty Liver Disease (NAFLD) Spectrum", by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates/figures.

research for numerous diseases. In the case of NAFLD, these modifications include histone methylation, a key mechanism in the regulation of lipogenic gene expression that impacts the accumulation of lipids necessary for NAFLD progression (Figure 2) (Juanola et al., 2021). Histone methylations are generally repressive, and removal by histone demethylases generally activates gene expression. The influence of histone demethylases on the development and progression of NAFLD is currently not fully understood, and understanding the regulation of repressive methylations by histone demethylases is key to furthering our understanding of NAFLD progression and its epigenetic contributors.

Epigenetic modifications have been of considerable scientific and medical interest and have become well-established as contributing factors in the diagnosis and treatment of NAFLD (Sodum et al., 2021). The effect of epigenetic regulation on NAFLD raises a number of questions but also presents an opportunity for a new avenue of therapeutic options; epigenetic modifications are, for the most part, reversible or adjustable and are proven drug targets (Majchrzak-Celińska et al., 2021).

This review explores the current research into the specific histone demethylases, lysine demethylase 7A (KDM7A), Jumonji domain-containing histone demethylase 2B (JMJD2B), and plant homeodomain finger 2 (PHF2), that have so far been implicated in the development and progression of NAFLD. The relationship between these histone demethylases and their relevant target genes is outlined in Figure 3. I summarize our current understanding of histone demethylases involved in the development and progression of NAFLD and suggest areas to direct future research.

DISCUSSION / LITERATURE REVIEW

Lysine Demethylase 7A (KDM7A)

KDM7A is a Jumonji-containing histone demethylase and plant homeodomain finger protein that has been implicated in the positive expression of genes associated with NAFLD progression (Kim et al., 2021). As a dual histone demethylase, it removes the repressive di-methylation marks histone 3 lysine 9 dimethylation (H3K9me2) and histone 3 lysine 27 dimethylation (H3K27me2) to form a mono-methylated state, H3K9me and H3K27me, respectively (Kim et al., 2021). In Kim et al. (2021), overexpression of KDM7A resulted in an increase in KDM7A localization at the promoter of diacylglycerol O-acyltransferase 2 (*DGAT2*) and a subsequent decrease in H3K9me2 and H3K27me2 within the promoter region. The authors observed an increase in *DGAT2* mRNA and protein alongside an increase in triglyceride (TG) levels, indicating an increase in hepatic steatosis; they observed the opposite with the knockdown of *KDM7A*. Finally, Kim et al. (2021) artificially inhibited *DGAT2* expression in *KDM7A*-overexpressing cells, and despite an increase in KDM7A, they observed normal TG levels. They concluded that KDM7A demethylates H3K9me2 and H3K27me2 from the histones that wrap the promoter sequence of *DGAT2*, resulting in an increase in *DGAT2* gene expression and a subsequent increase in the TG accumulation characteristic of NAFLD (Kim et al., 2021).

DGAT2 has been implicated as a controlling enzyme for TG homeostasis through catalysis of the final step of TG synthesis. It consumes DNL-produced fatty acids and catalyzes the final step in the esterification reaction that combines fatty acyl-CoA and diacylglycerols (DAGs) to form TGs, thereby increasing NAFLD (Kim et al., 2021). The increased expression of *DGAT2* as a result of *KDM7A* overexpression leads to greater formation of TGs and

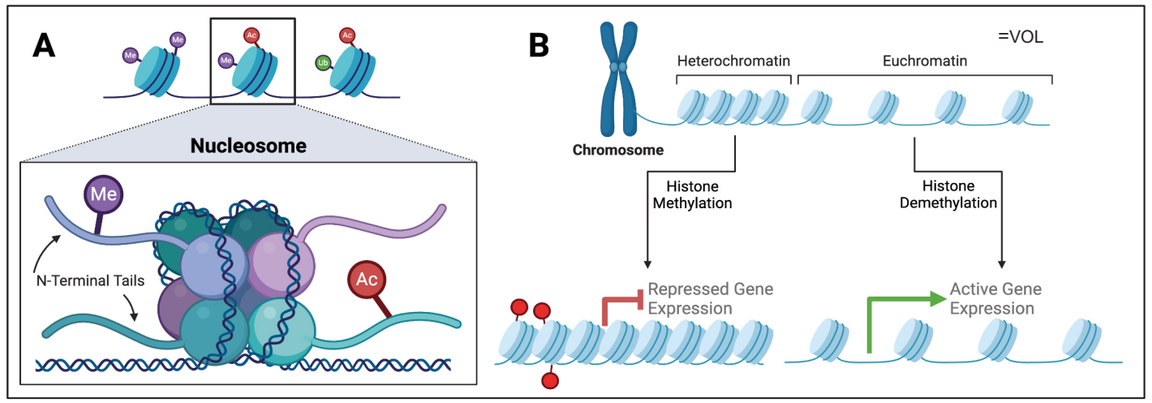

Figure 2 *Histone modifications and the effect on chromatin accessibility.* **A** Addition of functional groups to histone tails within the histone octamer that comprises the core of a nucleosome. **B** Methylation of the histone tails encourages heterochromatin formation and subsequently represses gene expression. Removal of these methylations by histone demethylases promotes a transition from heterochromatin to euchromatin and subsequently activates gene expression. Image created with BioRender.com.

subsequent lipid accumulation in hepatocytes. The incorporation of free fatty acids into TGs could indicate a protective mechanism against the detrimental effects of lipotoxicity caused by the accumulation of saturated fatty acids (SFAs) (Kim et al., 2021).

Jumonji Domain-Containing Histone Demethylase 2B (JMJD2B)

Liver X receptor α (LXRα) is a master transcription factor that regulates not only its own expression, but also lipogenic genes and the transcription factors sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP) (Kim et al., 2020). These genes include those responsible for DNL and cholesterol and glucose metabolism, which implicates LXRα in the development of a number of metabolic disorders (Grønning-Wang et al., 2013). LXRα has been shown to be rapidly upregulated by insulin, which aligns with ensuring that excess acetyl-CoA is converted to fatty acids and then TGs. The relationship between LXRα and the histone demethylase JMJD2B will be discussed here with respect to its influence on lipogenic gene expression. However, it is important to note that the activity of LXRα is more complex and involves the influence of ligands, pioneer factors, coregulators, and post-translational modifications (Grønning-Wang et al., 2013). Kim et al. (2020) showed that upon activation, LXRα recruits JMJD2B to the liver X receptor response element (LXRE) of the sterol regulatory element binding transcription factor 1 (*SREBF1*), fatty acid synthase (*FASN*), acetyl-CoA carboxylase (*ACC1*), and stearoyl-CoA desaturase 1 (*SCD1*). Subsequently, they tested the demethylation of histones within these LXREs via overexpression of *JMJD2B*. This enhanced the mRNA expression of these LXRx target genes as well as the protein levels of LXRα, SREBP1c, and fatty acid synthase (FAS). Additionally, knockdown of *JMJD2B* decreased measured mRNA and protein expression (Kim et al., 2020).

The gene *SREBF1* and its product SREBP-1c are essential in hepatic lipid metabolism (Kim et al., 2020). With LXRα and ChREBP, SREBP-1c regulates the expression of *FASN*, *ACC1*, *SCD1*, and *DGAT2* through binding to the sterol regulatory element (SRE) within the promoter regions of these genes. The subsequent lipogenesis, desaturation of SFAs, and esterification of DAG each contribute to the accumulation of lipids characteristic of NAFLD (Figure 3). Kim et al. (2020) measured the recruitment of LXRα and JMJD2B and the enrichment of H3K9me2 and H3K9me3 within the LXRE of the *SREBF1* promoter. LXRα activation and *JMJD2B* overexpression led to reduced H3K9me2 and H3K9me3 enrichment and coincided with an increase of JMJD2B and LXRα recruitment. Similarly, an increase in TG, *SREBF1* mRNA, and SREBP-1c protein were observed alongside an increase in hepatic lipid droplets. Kim et al. (2020) concluded that LXRα and JMJD2B work in tandem to regulate the expression of the lipogenic genes that contribute to the development and progression of NAFLD.

Plant Homeodomain Finger 2 (PHF2)

PHF2 is a member of the KDM7 histone demethylase family that has been linked to hepatic glucose metabolism and pro-inflammatory response *in vitro*. However, research into its physiological role is still in its infancy (Bricambert et al., 2018). In mice, Bricambert et al. (2018) established that PHF2 functions to remove the repressive histone methylation H3K9me2 within the promoter regions of its target genes and that PHF2-mediated demethylation of ChREBP-regulated gene promoters including NF-E2-related factor 2 (*Nrf2*), *SCD1*, *FASN*, *ACC*, and *DGAT2* acts to protect the liver from the lipotoxic effects resulting from an accumulation of pathogenic lipids. They showed that PHF2 was co-recruited with and dependent on ChREBP in order to bind to the carbohydrate response element (ChoRE) region of the *SCD1* promoter in response to stimulation by glucose. Additionally, they showed that *PHF2* knockout and overexpression led to a respective increase and decrease in H3K9me2 presence within the same promoter region, as well as a subsequent decrease and increase in chromatin accessibility and resultant DNL and TG levels. These results, together with an observed increase in liver TGs and DAG, indicate that *PHF2* overexpression favors the development of NAFLD (Bricambert et al., 2018).

Bricambert et al. (2018) discovered that *PHF2*-overexpressing mice had a significantly increased ratio of monounsaturated fatty acids (MUFA) to SFA compared to their controls. The previously established relationship between PHF2 and enhanced SCD1 expression explains the desaturation of SFAs to increase the proportion of MUFAs (Bricambert et al., 2018). SFAs are a primary factor influencing lipotoxicity, and *in vitro* studies showed that a dose-dependent introduction of MUFAs to cell cultures inhibits SFA-induced apoptosis (Nolan & Larter, 2009). Therefore, PHF2-driven expression of *SCD1* and the resultant conversion of SFAs to MUFAs may constitute a protective mechanism against the lipotoxic effects of SFA accumulation (Bricambert et al., 2018). Despite a greater accumulation of DAG and TG under PHF2 overexpression, hepatocytes were protected from SFA-induced insulin resistance and inflammation (Bricambert et al., 2018).

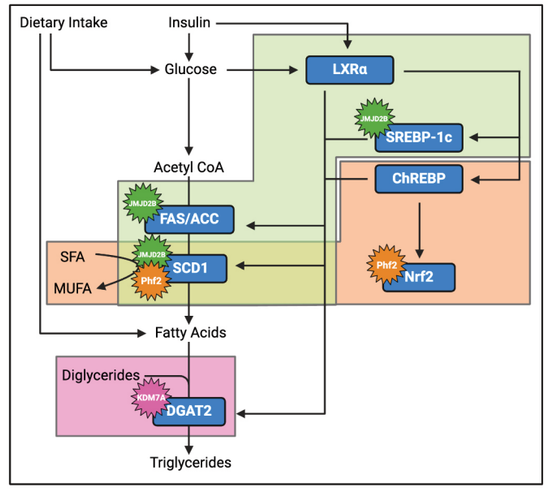

Figure 3 *Hepatic fatty acid metabolism and scope of reviewed research.* Summary of the scope of research conducted by Bricambert et al. (2018) in orange, Kim et al. (2020) in green, and Kim et al. (2021) in pink. Relevant histone demethylases are represented as the stars attached to their respective target genes. Image created with BioRender.com.

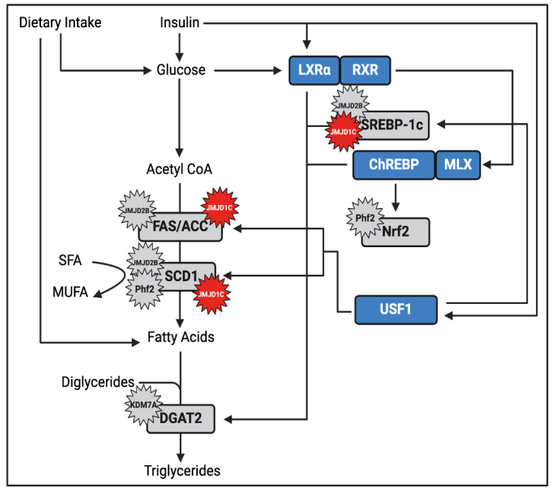

Figure 4 *Proposed overview of histone demethylase activity involved in the development and progression of NAFLD.* Summary of studies conducted by Bricambert et al. (2018), Kim et al. (2020), and Kim et al. (2021) with the addition of heterodimer partners RXR and MLX, transcription factor USF1, and histone demethylases JMJD1C. Image created with BioRender.com.

Bricambert et al. (2018) observed a higher mitochondrial oxidative capacity alongside *PHF2* overexpression. However, they did not observe the expected concomitant increase in reactive oxygen species (ROS). As an increase in ROS would lead to oxidative stress and contribute to lipotoxicity, these results suggested an additional protective mechanism of PHF2 on the progression of NAFLD. In response to glucose stimulation, Bricambert et al. (2018) found that PHF2 and ChREBP were co-recruited to the promoter of *Nrf2*, expressing antioxidant proteins and preventing the progression into fibrosis. *PHF2* overexpression in both wildtype and *Nrf2* knockout mice showed an increase in hepatic TGs and a greater MUFA/SFA ratio, which did not result in any pro-inflammatory responses. However, in the absence of *Nrf2*, *PHF2* overexpression promoted the progression into fibrosis and an increase in apoptotic cells and hepatic fibrotic areas (Bricambert et al., 2018).

Future Directions

The studies by Bricambert et al. (2018), Kim et al. (2020), and Kim et al. (2021) provide insight into the regulatory mechanisms of specific demethylases with respect to the progression of NAFLD. The genes identified in these studies, *SCD1*, *FASN*, *ACC*, and *DGAT2*, have been implicated in the desaturation, lipogenesis, and esterification of fatty acids responsible for the accumulation of lipids in hepatocytes. Figure 4 is a modified version of Figure 3, which indicates potential future research areas in histone demethylase activity and the development and progression of NAFLD. As shown in Figure 4, I suggest the influence of the formation of LXRα as well as ChREBP heterodimers and the contribution of the transcription factor upstream stimulating factor 1 (USF1) in partnership with the Jumonji domain-containing histone demethylase 1C (JMJD1C) on lipogenic gene promotion.

LXRα forms a heterodimer with the retinoid X receptor (RXR) in order to bind to the LXREs of its target genes (Figure 4) (Czimmerer & Nagy, 2023). RXR has been shown to remain bound to a relevant response element and, in the absence of a ligand, interacts with corepressors to downregulate gene expression (Czimmerer & Nagy, 2023). With the introduction of a ligand, corepressor-coactivator exchange occurs to upregulate gene expression. In the case of heterodimer formation with LXRα, the contribution of RXR is ligand-dependent, and free fatty acids constitute a relevant category of RXR ligands (Czimmerer & Nagy, 2023). I suggest further research into this relationship and its influence on lipid accumulation and NAFLD.

ChREBP undergoes post-transcriptional modifications in response to glucose stimulation, relocalizing to the nucleus where it forms a heterodimer with Max-like protein X (MLX) and binding ChoREs within its target genes (Yu et al., 2023). While this has not yet been examined with respect to NAFLD, a recent study has implicated its expression in the development of hepatocellular carcinoma (HCC). An increased expression of MLX is observed in HCC, and *in vivo* knockout of *MLX* inhibits cell proliferation and tumorigenesis (Yu et al., 2023). MLX not only responds to glucose stimulation, but it also is necessary in upregulating the lipogenic gene targets in partnership with ChREBP. It is conceivable that the regulation of MLX also contributes to NAFLD progression and needs to be considered when understanding the implications of the histone demethylases and subsequent ChREBP activity.

USF1 encodes a transcription factor whose activity contributes to the regulation of insulin-responsive genes and lipogenic enzymes (Viscarra & Sul, 2020). A relationship between USF1 nd JMJD1C is required to increase chromatin accessibility of lipogenic genes, as dysregulation of JMJD1C function resulting in the erroneous activity of USF1 is linked to hepatosteatosis, insulin resistance, and type 2 diabetes (Viscarra & Sul, 2020). JMJD1C, in the context

of hepatic lipogenesis, exclusively interacts with USF1 and, in response to insulin, is recruited to the promoters of lipogenic genes *FASN*, *ACC*, *SCD1*, and *SREBPF1* (Viscarra & Sul, 2020). Demethylation of H3K9me2 within these gene promoters increased chromatin accessibility that increased gene expression and subsequent lipogenesis. Furthermore, *JMJD1C* knockout resulted in infertility in C57BL/6J mice suggesting additional cellular functions in the body that could prevent it from being a target for NAFLD treatment (Viscarra et al., 2020). With respect to NAFLD and the effects of histone demethylases on gene regulation, it is imperative that we develop our understanding of the histone demethylases involved in order to create effective treatment options for this disease.

CONCLUSIONS

NAFLD affects a considerable number of people globally and is continuing to increase in prevalence every year. The lack of current treatment necessitates an understanding of the disease progression and contributing factors in order to understand the molecular "big picture" with a goal of developing new therapeutic methods. This review has explored the effects of histone demethylases on NAFLD development and progression and their suitability as potential drug targets. The histone demethylases KDM7A, JMJD2B and PHF2 and their partnership with master transcription factors LXRα, ChREBP, and SREBP-1c in their regulation of lipogenic genes *FASN*, *ACC*, *SCD1*, and *DGAT2* make these attractive targets. Overall, I conclude that these lipogenic genes, which function to increase lipid accumulation that results in NAFLD or act to protect from adverse effects of lipotoxicity once NAFLD has been established, may be suitable therapeutic targets. I have also proposed epigenetic interactions which may impact NAFLD that have not yet been tested; the effects and regulation of the functionally required heterodimer partners of both LXRα and ChREBP as well as the contribution of the transcription factor USF1 and histone demethylase JMJD1C on NAFLD development and progression. These present avenues for future research into understanding the progression of NAFLD. Developing our knowledge of the interplay between these elements can only aid in the advancement of therapies designed to treat NAFLD and benefit in the overall health of the global population.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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Framing the Canadian housing crisis: A discourse analysis of parliamentary debates

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ABSTRACT This study explores the political framing of the housing crisis in Canada through a discourse analysis of parliamentary debates. Drawing on a qualitative content analysis of one month of Hansards in March 2023, it examines how Canada's main political parties frame the source of the housing crisis. The research identifies six dominant frames, with excessive federal spending and excessive bureaucracy emerging as the most prominent. These frames reflect the polarized nature of political discourse and the strategic allocation of blame across different levels of government. By linking these findings to literature on political framing and multi-level governance, the study highlights how competing narratives shape public policy in a decentralized federation. The results suggest that such framing may hinder the development of cohesive and effective housing policy.

INTRODUCTION

As housing prices and rental costs climb across Canada, public pressure has called for increased government action. In response, federal, provincial, and municipal interventions have been introduced to alleviate affordability and supply challenges, including the use of public land for affordable housing, rental development loan programs, and changes to mortgage regulations. Despite these measures, housing affordability remains a persistent challenge. This study seeks to understand the political characterization of the origin of this problem. Drawing on a qualitative content analysis of one month of Hansards in March 2023, it examines how Canada's main political parties frame the source of the housing crisis.

The study begins with an overview of Canada's housing challenges and a discussion of the conceptual framework and methodology. A central focus is the construction of a crisis frame and its significance for public policy decision-making. The findings note excessive federal spending and excessive bureaucracy as the dominant frames. Particularly among the Liberal Party of Canada and Conservative Party of Canada, the study also finds notable influence of partisan affiliation in shaping the discourse of the crisis. It concludes with a discussion of these findings and their broader policy implications for multi-level governance.

LITERATURE REVIEW

The housing crisis in Canada presents various ideological, regional, and social factions in public policy. In all cases, there is widespread recognition of the unaffordability of housing for renters and home buyers alike (Nistor & Reianu, 2018; Singh, 2022). Among Organisation for Economic Co-operation and Development countries, Canada's housing market is among the most unaffordable (Zhu, 2023). While housing prices increased by over 230% between 2000 and 2019, median income in Canada only rose by 74% (Zhu, 2023, p. 1861). Zhu (2023) notes that housing policies have focused on achieving a supply-demand equilibrium by discouraging foreign investment in the Canadian market. In a study on media framing and the rise of housing nationalism in Canada, Lauster and Bergmann (2023) similarly identify foreign investment as a primary threat to the housing market. A 2023 Fraser Institute report broadens the discussion to include population growth and the completion of housing units. In the report, Filipowicz (2023) argues that the increasing disparity between housing demand (population growth) and housing supply (housing completions) has led to significant implications for housing affordability nationwide. Reflecting key considerations surrounding housing in Canada, I anticipated these frames would play a significant role in the housing discourse among Canadian Members of Parliament.

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Despite considerable media attention on the housing crisis, scholarly work on how Canadian Members of Parliament frame the issue is limited. While the literature identifies multiple causes of the housing crisis, it remains unclear whether political discourse reflects these explanations or emphasizes alternative narratives. To address this gap, this study draws on the research of White and Nandedkar (2021), who examine the political discourse associated with the housing crisis in New Zealand. They argue that the political framing of housing as a crisis is ideologically siloed and that the dominant frame used to explain the issue is inefficiency within local government, specifically the restricted supply of land and overbearing regulations (White & Nandedkar, 2021). Notably, they demonstrate that New Zealand's governing party resisted framing the housing issue as a crisis but deployed significant resources in response to it (White & Nandedkar, 2021). In focusing my study on partisan and ideological divides in the House of Commons, I also seek to determine whether the governing Liberal Party of Canada offers similar resistance.

CONCEPTUAL FRAMEWORK

This study employs a content analysis of Hansards to ascertain the dominant frames of the housing crisis. A content analysis allows researchers to test theoretical concepts and clarify textual data into content-related categories (Elo & Kyngäs, 2008). The objective is to achieve a condensed description of the phenomenon under study, yielding categories that describe it (Elo & Kyngäs, 2008). In this study, a content analysis facilitates the identification of key themes and patterns in the discourse surrounding the housing crisis, particularly its dominant frames. Framing is an approach in research seeking to understand how messages are created and disseminated (White & Nandedkar, 2021). White and Nandedkar (2021) emphasize the importance of counterframing, which refers to efforts to sway public opinion, often involving the attribution of political blame. The debates associated with crises are especially relevant to the issue of housing in Canada. White and Nandedkar (2022) write that crises are ideologically inclined assertions of truth that position a problem and prompt a related policy response. Heslop and Ormerod (2020) add that crises create opportunities for new political narratives. In this context, I focus on how different political parties adopt the crisis frame when discussing housing issues in Canada and how the Conservative Party of Canada may counterframe the housing crisis to assign responsibility to the governing party.

METHODOLOGY

The primary data source for this study is one month of Hansards. A publication search using the keyword "housing" on the House of Commons Hansard database was conducted to identify debates among Members of Parliament related to the housing crisis during March 2023. The month of March 2023 was selected for analysis due to the release of Budget 2023 at the month's end, with the assumption that debates on significant policy matters would intensify during this period. An initial search yielded 98 debate transcripts: 55 were rejected for falling outside the scope of this study, either for lacking mention of the housing crisis or failing to discuss the source of the problem, and the remaining 43 transcripts were combined in a single file and imported into NVivo.

Drawing on the work of White and Nandedkar (2021), I conducted a content analysis of the 43 parliamentary Hansards to investigate how Canada's main political parties frame the source of the housing crisis. The coding scheme was developed inductively, which enabled the recognition of emergent themes and patterns within the data. This iterative process ensures that the analysis is reflective of the framing of the housing crisis by different political parties. Following an initial scan of the data, six coding categories describing the source of the housing crisis were identified. These categories were used to conduct a secondary review of the data, which focused on the frequency of occurrence and the political affiliation of the speaker. Instances where a coding category was mentioned multiple times within a single transcript were treated as a singular occurrence. Table 1 outlines the coding categories and definitions used in this study.

Table 1. Coding categories and definitions.

Code	Code Name / Label	Definition
C1	Material or labour shortages	Frame portraying a situation where there is a lack of necessary materials or labour force, hindering the progress of housing construction or development projects
C2	Individual or foreign investment	Frame portraying investment made by domestic or foreign individuals or entities in the housing market for profit
C3	Insufficient public-sector housing developments	Frame portraying a condition where the government's (all levels) efforts in constructing or providing housing units fall short of addressing the housing demand
C4	Excessive federal spending	Frame portraying the excessive or wasteful spending of the federal government, potentially leading to inflationary pressures
C5	Excessive bureaucracy	Frame portraying the regulations or procedures imposed by government (all levels) as overly complex, hindering efficient housing development or investment
C6	High taxes	Frame portraying a situation where the level of taxes imposed on housing-related activities is too high

RESULTS

An examination of Hansards during March 2023 indicates the prominence of two primary frames in the discourse surrounding the origin of the housing crisis. Table 2 presents the frequency of occurrence and the percentage of debates of each frame. In a sample of 43 transcripts, the topic of excessive federal spending was raised 18 times, accounting for 34.6% of the debates, while references to excessive bureaucracy were made 24 times, representing 46.1% of the debates.

To broaden the scope of these findings, I investigate how the frames are ideologically distributed. Table 3 presents the frequency of occurrence of each frame among Canada's main political parties (LPC: Liberal Party of Canada, CPC: Conservative Party of Canada, NDP: New Democratic Party, BC: Bloc Québécois). While the Green Party is one of the main political parties, there were no mentions of the housing crisis from its representatives in the Hansards during March 2023. I find that all six coding categories were represented as frames of the sources of the housing crisis by at least one of the four political parties. However, the prominence of the two dominant frames, excessive federal spending and excessive bureaucracy, suggests noteworthy

ideological distinctions. Excessive federal spending is exclusively raised by the Conservative Party and is typically associated with claims of reckless spending and fiscal deficits leading to heightened inflation. I acknowledge that the validity of these claims falls outside the scope of this study and focus instead on how the source of the housing crisis is framed in this context. For example, Marc Dalton (Pitt Meadows-Maple Ridge, CPC) notes:

> "In greater Vancouver, the dream of buying a home or even renting an affordable place has become a nightmare under the Liberal government. The Liberals do not care. They will not take any responsibility for the mess they have created. Reckless spending and the irresponsible doubling of our national debt have lit an inflationary fire. After eight years under the Prime Minister, the price of housing has skyrocketed."

Table 2. Frequency of occurrence of each frame.

Code	Frequency of Occurrence	Percentage of Debates
C1	1	1.9%
C2	1	1.9%
C3	5	9.6%
C4	18	34.6%
C5	24	46.1%
C6	3	5.7%

Table 3. Frequency of occurrence of each frame by political party.

	Frame	C1	C2	C3	C4	C5	C6
Political Party	LPC	0	1	0	0	6	0
	CPC	0	0	1	18	17	3
	NDP	0	0	2	0	1	0
	BC	1	0	2	0	0	0

Focusing on "reckless spending", Dalton associates the Liberal Party's governance with increases in housing prices. Todd Doherty (Cariboo-Prince George, CPC) draws a similar conclusion:

> "The average rate for a studio apartment in my home province of British Columbia is $2,200, and mortgage rates are doubling, all because of the Prime Minister's out-of-control inflationary spending."

Finally, Dominique Vien (Bellechasse-Les Etchemins-Lévis, CPC) reiterates this narrative and specifically categorizes the situation as a crisis:

> "Paying their rent has become a headache for Canadians. The monthly cost of a two-bedroom apartment has doubled in the 10 largest Canadian cities since 2015. This phenomenon was created by this Prime Minister with his out-of-control spending, which has impacted inflation… Will the Prime Minister finally accept responsibility for the crisis he has created?"

An examination of potential regional differences in framing for renters relative to home buyers was inconclusive. Instead, the Conservative Party has engaged in consistent pan-Canadian messaging. By linking housing affordability directly to what they describe as "reckless spending" and "out-of-control inflationary spending", these Members of Parliament construct a narrative that seeks to portray the Liberal Party as responsible for exacerbating the housing crisis. As the discourse often includes criticisms of the current government or insinuates that the Conservative Party could address the housing issue more effectively, I suggest that this framing is also strategically employed for political purposes. It points to the Conservative Party's ideological stance on fiscal responsibility and reflects a broader strategy to discredit the current government's economic policies. The frame of excessive bureaucracy, typically at the municipal level, is discussed substantially among both the Liberal Party and Conservative Party relative to the other frames. Pierre Poilievre (Carlton, CPC) notes:

> "The average required down payments, rents and mortgage payments have doubled under the Prime Minister. His inflationary policies have made life worse, and his gatekeeping friends prevent housing construction. Will the Prime Minister announce in tomorrow's budget serious penalties for the gatekeepers that drive up housing prices so that hard-working Canadians can have homes they can afford?"

Apart from one reference to restricting foreign direct and domestic investment in the housing market, the Liberal Party exclusively attributes the housing crisis to excessive bureaucracy. In response to Conservative Party claims, Justin Trudeau (Papineau, LPC) remarks:

> "I recognize, as this government recognizes, that we need to work with municipalities to help them change zoning laws, to help them accelerate their permitting processes and to create more opportunities to build affordable homes for Canadians across the country, whereas he sits back and attacks them and proposes absolutely nothing. We are stepping up with $4 billion to accelerate the supply of homes across this country. We will continue to invest and work with partners instead of picking fights with everyone and hoping that it all settles itself."

Prime Minister Trudeau advocates for collaboration with municipalities and highlights efforts to increase government funding to accelerate affordable housing construction. By emphasizing the actions and initiatives undertaken by the Liberal government to address housing affordability, the Prime Minister avoids directly attributing blame to municipalities or acknowledging the crisis, which could be perceived as an evasion of responsibility. As with other social or economic policy matters, the politicization of the housing crisis is evident.

DISCUSSION

The findings of this study suggest that the dominant frames of the source of the housing crisis in Canada are excessive federal spending and excessive bureaucracy. While the literature on the source of the housing crisis focuses primarily on foreign investment and population growth (Lauster & Bergmann, 2023; Filipowicz, 2023), political discourse often emphasizes government inefficiency and regulatory barriers. This suggests a disconnect between the political framing of the crisis and the

factors that contribute to it. However, these findings are consistent with the research of White and Nandedkar (2021) on the housing crisis in New Zealand. Their characterisation of the political framing of the housing crisis as ideological and their presentation of inefficiency within local government reflect the debate among Canadian Members of Parliament in the House of Commons during March 2023. Additionally, these findings align with their observation that, despite not explicitly labelling the situation as a crisis, the governing party has allocated significant resources to address housing challenges (White & Nandekar, 2021). The Liberal Party mentioned the source of the housing crisis 84% less than the Conservative Party (Figure 3) yet was consistent in highlighting the government's efforts to address the issue. This implies a discrepancy between the conceptualization of the issue in political discourse and the measures implemented by policymakers.

A key consideration in this study is the politics of apportioning blame in a decentralized federation. White and Nandekar (2021) describe how counterframing is used to attribute political blame. In a decentralized federation, assigning responsibility for political outcomes is especially complex because power is distributed across various levels of government and involves shared responsibilities among actors at different tiers (Maestas et al., 2008). Housing is a shared responsibility among the federal, provincial, and territorial governments, but municipal governments also contribute to the housing regulatory framework through their control over zoning and construction. Maestas et al. (2008) note that in multi-level governance systems, political actors are motivated to deflect blame towards other levels. While the Conservative Party attributes blame to the governing Liberal Party, the Liberal Party shifts this blame to municipalities. Culter (2008) suggests that these intergovernmental challenges are facilitated by the lack of concurrent elections. Without concurrent elections, the governing party can transfer blame to another level of government knowing that it faces no immediate electoral pressure to respond (Culter, 2008). I would add that the lack of integration of political parties across all levels of government in Canada facilitates the vertical attribution of blame because the blame-assigning party faces few political repercussions. Without a shared party affiliation (i.e., municipalities do not operate under the same party structures as provincial or federal governments), responsibility is more easily deflected. Considering the diverse priorities and leadership of Canada's municipalities, widespread resistance to the federal framing of the housing crisis ahead of the 45th Canadian federal election is unexpected or will likely not overshadow the influence of the federal government. At the same time, Culter (2008) argues that efforts to deflect blame are countered by opposition parties assigning as much blame as possible to the governing party. This may explain why the Conservative Party raised the issue of excessive bureaucracy 64% more times than the Liberal Party, often blaming the municipalities while criticizing the Liberal Party for its perceived inaction regarding them. The Conservative Party has counterframed the Liberal Party's narrative by shifting the focus from municipal responsibility to federal government inefficiency. This observation underscores the complexity of assigning political responsibility in a decentralized federation, particularly in the context of the housing crisis.

LIMITATIONS

In conducting this study, two primary limitations should be acknowledged. First, the reliance on Hansards from a single month may restrict the generalizability of the findings to broader temporal contexts. This may overlook potential fluctuations in the discourse outside the selected timeframe. Second, the findings may not offer a comprehensive understanding of the source of the housing crisis because other forms of political communication not studied (e.g., speeches, press releases, interviews) play a significant role in shaping political discourse. Future research could track the evolution of the framing of the housing crisis and examine different forms of political communication.

CONCLUSIONS

This study has examined the framing of the source of the housing crisis among Canada's main political parties using a content analysis of 43 Hansards during March 2023. It emphasizes the role of partisan affiliation in shaping political discourse and the complexity of assigning political responsibility in a system of multi-level governance. I identify six frames and find the prominence of two: excessive federal spending and excessive bureaucracy. The study then emphasizes the politicization of the housing crisis and the contestation of blame among the Conservative Party and Liberal Party. Particularly in a decentralized federation, the shifting narratives surrounding the housing crisis reflect the challenges in achieving consensus on effective policy solutions.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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Utilizing trauma-informed approaches in prisons for federally sentenced women: Challenges and recommendations

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ABSTRACT This paper examines literature, policy documents, and government reports on trauma-informed care in federal prisons for incarcerated women and the difficulties of providing such care in a carceral environment. Incarcerated women represent a minority of incarcerated people in Canada, and they typically have increased rates of trauma compared to incarcerated males, which impairs their ability to participate in correctional programs fully. Many researchers recommend implementing trauma-informed approaches in correctional settings, and Correctional Service Canada (CSC) has attempted to do so for federally sentenced women by adhering to principles outlined in *Creating Choices*, a report created to influence the care of incarcerated women. Despite CSC's efforts to use trauma-informed approaches, the non-therapeutic prison environment and the power dynamics between prisoners and staff make it impossible to provide trauma-informed care in prisons, as women are often re-traumatized in prison due to common security practices such as strip searches. Therefore, this paper offers policy change recommendations, including eliminating strip searches and providing correctional programming led by external treatment providers, that would minimize the harm women experience while incarcerated. However, given the inherent harms associated with prison that cannot be addressed through reform, allowing women to serve their sentences in the community is optimal and necessary to reduce the use of imprisonment and truly provide them with trauma-informed care.

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INTRODUCTION

Women constitute a small proportion of those incarcerated in Canadian federal penitentiaries[1], with recent statistics highlighting that 6% of federal offenders in Canada are women (Corrections Service Canada [CSC], 2024). Given the small percentage of women in prison, their unique treatment needs are not sufficiently addressed by correctional programming. One example of their unique needs that may be ignored is their histories of trauma. Although trauma is defined differently across studies, the BC Trauma-Informed Practice Guide defines it as "… experiences that overwhelm an individual's capacity to cope" (B.C. Centre of Excellence for Women's Health [BCCEWH], 2013, p.6), listing examples like child abuse and experiencing violence. Scholars have argued that prisons do not meaningfully address incarcerated women's trauma histories (Parkes, 2016) and they have critiqued CSC, the federal correctional system, for its use of Creating Choices[2] as a guide for women's correctional care because, despite including trauma-informed principles, it is difficult to implement them in a punitive prison environment (Hannah-Moffat, 1995; Hayman, 2006; Pollack, 2009) that can re-traumatize women (Hutchison, 2020). The inability to adhere to the trauma-informed principles in Creating Choices is concerning, given the high trauma rates among incarcerated populations (Bodkin et al., 2019).

In addition to high trauma rates among incarcerated individuals compared to the general population (Bodkin et al., 2019), research suggests that rates of trauma are higher among female offenders than male offenders (Covington & Bloom, 2007; Martin et al., 2015; Tam & Derkzen, 2014). Martin et al. (2015) found that federally incarcerated women in Canada had higher rates of childhood trauma than federally incarcerated males, and Covington and Bloom (2007) noted that female offenders experience more physical/sexual abuse than male offenders. Gender disparities appear to be more prevalent among rates of sexual trauma and childhood sexual abuse (Bodkin et al., 2019; Power et al., 2016; Tam & Derkzen, 2014). Overall, rates of trauma among incarcerated women are staggering, with over 80% experiencing physical violence and almost 70% experiencing sexual violence (Office of the Correctional Investigator [OCI], 2021).

[1] Individuals who are federally sentenced in Canada receive a custodial sentence of two years or longer
[2] Creating Choices is the report that informs CSC's care of federally sentenced women – it will be discussed in detail later in the paper

Women offenders who have experienced trauma often react with internalizing behaviours (Miller & Najavits, 2012); as such, trauma exposure is often associated with women experiencing PTSD symptoms (Miller & Najavits, 2012; Tam & Derkzen, 2014), emotional dysregulation (Covington, 2008), substance abuse (Covington, 2008; Tam & Derkzen, 2014), suicide attempts (Clements-Nolle et al., 2009), and self-injurious behaviour (Tam & Derkzen, 2014). Trauma exposure is also related to increased offending (Tam & Derkzen, 2014), institutional misconduct (Martin et al., 2015), and violence among women offenders (Trabold et al., 2015). Lastly, trauma may affect women's engagement in correctional programs by impairing therapeutic alliances with staff and/or the therapeutic environment (Covington, 2008).

Given the negative effects associated with experiencing trauma, many researchers have recommended implementing trauma informed approaches in prisons (Bodkin et al., 2019; Miller & Najavits, 2012). However, given the punitive nature of prisons (Hayman, 2006; Pollack, 2009), it may be difficult to use trauma informed approaches when providing programming. As such, this research paper addresses the question: Can trauma-informed care be provided to women incarcerated in Canadian federal correctional institutions? To do so, trauma-informed care will be explained and linked to Creating Choices, a report that informs CSC's treatment of federally sentenced women. Then, critiques of Creating Choices will be considered, as well as features of the prison environment that make implementing trauma-informed approaches challenging. Although it is also necessary to provide trauma-informed services to incarcerated males, the focus of this paper is on women because Creating Choices was written for women and is the report used by CSC. This research question is best addressed by a narrative review, as it allows for an in-depth examination of the usefulness of trauma-informed approaches in prison that could inform future programming provided to women in the criminal justice system.

DISCUSSION/LITERATURE REVIEW

Trauma-informed and gender-specific approaches to treating women in custody

Given the high rates of trauma among incarcerated individuals, but especially among incarcerated women (Martin et al., 2015), many experts recommend implementing trauma-informed approaches in prisons (Bodkin et al., 2019; Miller & Najavits, 2012). Trauma-informed approaches are services that consider an individual's history of trauma to help clients feel safe and empowered to prevent re-traumatization; importantly, the service does not have to be focused on treating trauma to be trauma-i nformed (BCCEWH, 2013). When assessing an individual's history of trauma, strengths-based approaches that emphasize coping skills can be used to empower individuals (Fallot & Harris, 2001); however, trauma-informed approaches can be used even if someone has not admitted to experiencing trauma, as it is more about a treatment provider's way of interacting with clients rather than specific treatments (BCCEWH, 2013). For example, trauma-informed approaches emphasize a client's ability to make decisions about treatment at a pace they are comfortable with, as well as providers having open communication with clients

(BCCEWH, 2013). Utilizing trauma-informed approaches is important because of the negative impact trauma can have on program participation (Covington, 2008).

Researchers also highlight the need for correctional programming to meet incarcerated women's unique treatment needs (Covington & Bloom, 2007). The principles of trauma-informed approaches overlap with many tenets of gender-specific care, such as providing individuals with a safe environment and treating them with respect to prevent re-traumatization (BCCEWH, 2013; Covington & Bloom, 2007). Additionally, gender-specific services include addressing trauma (Gobeil et al., 2016) due to the high rates of trauma among incarcerated women. Thus, using trauma-informed approaches is in line with providing gender-specific care.

Preliminary evidence supports using trauma-informed, gender-specific services to treat women offenders. Gobeil et al.'s (2016) meta-analysis, once limited to methodologically rigorous studies, found that participation in gender-specific treatments reduced recidivism more than gender-neutral interventions among women offenders. Similarly, women offenders who received gender-responsive treatment had lower rates of PTSD than those who received mixed-gender treatment or no treatment (Messina et al., 2014). Moreover, the benefits of using trauma-informed approaches in prisons include reduced costs, reduced rates of adverse events in custody like seclusion (Miller & Najavits, 2012), and increased success among women reintegrating into the community upon release (Doherty et al., 2014).

Creating Choices and its connection to trauma-informed practice

Creating Choices (see Task Force on Federally Sentenced Women [TFFSW], 1990) proposed five principles to guide the treatment of incarcerated women. Each principle aligns with recommendations of trauma-informed approaches:

1. Empowerment

In Creating Choices, empowerment is discussed in the context of self-esteem. Because of the inequities women may experience, they may feel incapable of making their own decisions; empowering women allows them to make choices about their recovery (TFFSW, 1990). Empowering individuals is also a tenet of trauma-informed approaches (BCCEWH, 2013).

2. Meaningful and Responsible Choices

Like the prior principle, having women make their own decisions will empower them and help them make responsible choices upon release (TFFSW, 1990). Trauma-informed approaches also emphasize allowing individuals to make treatment choices (BCCEWH, 2013).

3. Respect and Dignity

This principle highlights the necessity for respectful relationships to encourage women to assume responsibility (TFFSW, 1990). Respectful relationships are also important in trauma-informed approaches to promote healing (BCCEWH, 2013).

4. Supportive Environment

This principle involves considering an individual's needs in treatment and creating a positive environment (TFFSW, 1990). Providing a supportive environment is essential in gender responsive treatment (Covington & Bloom, 2007) and trauma-informed care (BCCEWH, 2013).

5. Shared Responsibility

Under this principle, it is essential for numerous agencies like CSC, the government, and the community to empower and care for women (TFFSW, 1990) to help them take ownership of their actions. Although this principle is not directly connected to trauma-informed approaches, its intended outcome of empowerment aligns with trauma-informed approaches (BCCEWH, 2013).

Critiques of Creating Choices

Despite the authors of Creating Choices stating that prisons should not be used as a long-term solution, they recommended opening numerous prisons for women (TFFSW, 1990). As such, many scholars have critiqued Creating Choices for upholding incarceration as a solution to crime instead of questioning its use (Hayman, 2006). Although Creating Choices was intended to reform the approach to treating incarcerated women, it "… soon came to be viewed as a veil for re-legitimating a renewed form of punishment" (Maurutto & Hannah-Moffat, 2016, p. 177) and failed to create meaningful change for incarcerated women (Hannah-Moffat, 2013). With the opening of new women's prisons, scholars highlighted a shift to a more punitive carceral environment and away from using community resources (Maurutto & Hannah-Moffat, 2016).

Further, although community organizations helped develop Creating Choices, CSC's freedom to implement the principles without oversight has failed to improve the prison environment for women. The Canadian Association of Elizabeth Fry Societies (CAEFS) were involved in the Task Force that developed Creating Choices along with advocates, formerly incarcerated women, and other members of the public (CAEFS, 2022). Although involving community organizations seemed promising, CAEFS noted their recommendations were not implemented by CSC as intended (CAEFS, 2022). Further, the imprecise and undefined language in Creating Choices (Hayman, 2006) has allowed CSC to interpret words like empowerment (Hannah-Moffat, 1995) in a way that was not intended by Creating Choices. Lastly, there are concerns that Creating Choices failed to consider the realities of prison. For example, characterizing women's prisons as therapeutic ignores that they are punitive by nature (Hannah-Moffat, 1995). Also, the report failed to consider the challenges of implementing the principles in a punitive environment (Hannah-Moffat, 1995; Hayman, 2006; Pollack, 2009).

The prison environment: Violating Creating Choices and tenets of trauma-informed care

Although federal women's correctional facilities are intended to be rehabilitative, as illustrated by the principles of Creating Choices, the need to maintain control in prison (Miller & Najavits, 2012) makes it challenging to prioritize prisoners' needs (Hayman, 2006). The focus on punishment and control (Pollack, 2009) creates a coercive prison environment that makes it difficult for women to benefit from programs while incarcerated (Kilty, 2012; Pollack, 2009). Moreover, the OCI notes that despite Creating Choices, security-driven approaches are still predominately utilized in women's corrections (OCI, 2021). Ultimately, prisons do not promote healing: "They are sites of coercion, repression, and pain" (Hayman, 2006, p. 257).

Women may be re-traumatized by the prison environment and security practices. For example, loud noises and constant light exposure (Miller & Najavits, 2012) can be triggering for those who have experienced trauma. Furthermore, women may be traumatized by security practices (Covington & Bloom, 2007; Messina et al., 2014), such as strip searches (OCI, 2019). Formerly incarcerated women in Canada who had been strip-searched while residing in a Canadian provincial or federal prison explained that being strip-searched was a similar experience to being sexually assaulted (see Hutchison, 2020). These strip searches also brought up feelings of previous trauma, and the adverse effects of strip searches were worse for women who had previously been sexually victimized (Hutchison, 2020). In addition, women reported that practices such as random strip searches made them feel unsafe in prison (OCI, 2021).

The non-therapeutic prison environment and potential for re-traumatization violate principles of trauma-informed practice, including those outlined in Creating Choices. The coercive prison environment limits women's ability to make decisions about their treatment (Kilty, 2012; Pollack, 2009). Although programs cannot be forced onto prisoners, pressure exists to follow treatment recommendations to obtain parole release (Kilty, 2012). Coerced participation violates the principle of meaningful and responsible choices in Creating Choices, which emphasizes empowering women to make their own decisions (TFFSW, 1990). Moreover, in line with gender-responsive, trauma-informed care (BCCEWH, 2013; Covington & Bloom, 2007) and the Creating Choices supportive environment principle (TFFSW, 1990), it is crucial to provide a safe treatment environment to prevent re-traumatization, which prisons fail to do.

Re-traumatization also occurs because of power imbalances between staff and prisoners that make it difficult to have respectful relationships. For example, among correctional officers and prisoners (Messina et al., 2014), power imbalances make it challenging for women to refuse to be strip-searched because of potential repercussions (Hutchison, 2020). Strip-searching prisoners and searching their cells reinforces the hierarchy between staff and prisoners, thereby validating existing power imbalances (Balfour, 2018; Hutchison, 2020). Moreover, as officers often engage in coercive practices to maintain control, these practices can further strain their relationships with prisoners (Meško & Hacin, 2019). Power imbalances extend into relationships between prisoners and medical staff. It can be challenging to disclose information to people employed by the prison (Pollack, 2009) out of fear that their medical information will be used against them at parole hearings (Kilty, 2012). This fear creates barriers to forming therapeutic relationships (Kilty, 2012) and may impair women's ability to benefit from programs.

These power imbalances violate principles of trauma-informed practice, including those outlined in Creating Choices. In trauma-informed approaches, it is essential to have mutually respectful relationships between providers and clients to remove existing power imbalances (BCCEWH, 2013). The hierarchy between prisoners and staff does not allow mutually respectful relationships to form. It violates the principle of respect and dignity in Creating Choices (TFFSW, 1990), further highlighting the challenges of using trauma-informed approaches and adhering to the principles of Creating Choices in Canadian federal prisons.

CSC policies that violate Creating Choices and the tenets of trauma-informed care

Some of CSC's institutional practices violate trauma-informed approaches/Creating Choices principles. In a recent report, the Correctional Investigator of Canada, Dr. Zinger, reported two practices that violate these principles: the overly restrictive movement level system and the arbitrary strip-search protocol (OCI, 2019, 2021).

The excessively restrictive movement level system in place for maximum security women violates the principles of Creating Choices (OCI, 2019). This system has three levels, and to highlight its severity, individuals on level 1 are generally restrained while accessing institutional services (OCI,2019). Despite recommendations to remove this system, CSC has kept the system largely unchanged to manage women who are accessing services off their unit (OCI, 2019). This system is not in place at men's federal institutions, suggesting it is unnecessary and gendered (OCI, 2019). Further, West Coast Prison Justice Society (2020) has highlighted the unlawfulness of restrictive movement routines among incarcerated individuals.

Strip searches are also not in line with the principles of trauma-informed care. CSC has used a random strip search protocol in women's institutions that allows strip searches to be conducted randomly without reasonable cause (OCI, 2019). This protocol reinforces security instead of creating a supportive environment, as was recommended in Creating Choices (OCI, 2019). Routine strip searching is not trauma-informed and violates the principles of Creating Choices. The OCI notes "…security-driven practices like these often reproduce traumatic events and worsen symptoms of previous trauma" (OCI, 2021, p. 35) and a woman who was strip searched hundreds of times noted "it was emotionally degrading" (Hutchison, 2020, p. 168). Given these negative effects, the Standing Senate Committee on Human Rights (2021) has recommended CSC "… cease the use of routine strip searching of federally sentenced women" (p. 28).

Can trauma-informed care be provided for federally incarcerated women?

Despite CSC's efforts to provide gender-responsive programs (Allen & Wardrop, 2022), the non-therapeutic prison environment (Hayman, 2006; Pollack, 2009) and power imbalances between prisoners and staff (Messina et al., 2014) make it impossible to provide trauma-informed programming in prison. Despite Creating Choices being in place for over 30 years, there have been few improvements for incarcerated women (OCI, 2021). Although policies can be revised to minimize the harm women experience while incarcerated, prisons are inherently punitive (Hayman, 2006) and not set up for rehabilitation - no amount of reform can change this (Faith, 2000; Hannah-Moffat, 2013). As Parkes (2016) highlighted, "… making arguments that assume prisons are inevitable and simply seek[ing] to "reform" them can do harm" (p. 22).

Policy recommendations

1. Use community corrections approaches when available.

In the last decade, the Canadian federal women's prison population has increased by 32.5% (OCI, 2019), making it crucial to reduce the use of imprisonment (CAEFS, 2020). Further, as prisons are inherently punitive (Hayman, 2006), trauma-informed care should be provided in the community in a safe environment (BCCEWH, 2013). Thus, judges should impose community sentences, particularly for individuals who have experienced trauma.

2. Eliminate strip searches.

Strip searches are inherently coercive and can re-traumatize women (Hutchison, 2020; OCI, 2019). Many scholars and non-profit groups, both in Canada (CAEFS, 2020; Hutchison, 2020) and internationally (Human Rights Law Centre & Flat Out, 2024), have argued that CSC, and prisons more broadly, should eliminate the practice of strip-searching incarcerated women.

3. Provide correctional programming led by external treatment providers.

Services should be provided by providers outside of CSC (CAEFS, 2020). Research suggests that women have better experiences receiving treatment from external providers (Pollack, 2009). Additionally, external providers allow them to provide specialized services (Ervin et al., 2020) rather than relying on correctional staff who may not have the skills to run the program. There is also less of a power imbalance between external personnel and prisoners compared to prisoner-staff dynamics, in line with tenets of trauma-informed practice (BCCEWH, 2013).

CONCLUSIONS

The small proportion of federally sentenced women in Canada makes it harder to address their unique treatment needs. In comparison to male offenders, women offenders are more likely to have histories of trauma (Covington & Bloom, 2007; Martin et al., 2015; Tam & Derkzen, 2014). This trauma can have several consequences, including impairing program participation (Covington, 2008). Given the evidence supporting gender responsive, trauma-informed approaches (Gobeil et al., 2016), many researchers recommend using these approaches in prisons. However, the prison environment and CSC's policies are not conducive to providing trauma-informed care. Despite CSC's efforts to provide trauma-informed programming by adhering to Creating Choices, this care cannot be provided in prison. Although policy revisions can minimize the trauma women experience while incarcerated, the only effective way to deliver trauma-informed care is in a community setting. Accordingly, it is crucial to reduce the use of imprisonment by allowing women to serve their sentences in the community when available.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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4th Year PharmD Students' Perceived Preparedness to Prescribe in British Columbia

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ABSTRACT New regulations in British Columbia have authorized pharmacists to prescribe for minor ailments and contraception. It is unknown whether the current University of British Columbia's Entry-to-Practice Doctor of Pharmacy (E2P PharmD) Program has prepared its students for this expansion in prescribing. The current study aimed to assess 4th-year E2P PharmD students' perceived preparedness to prescribe as they transition from the role of students to newly graduated pharmacists and to identify if any changes need to be made to the current PharmD curriculum. The study used survey methodology and contained questions associated with preparedness to prescribe. Questions consisted of Likert scales, ranking, multiple response, and open-ended formats. The web-based survey was made available to 4th-year E2P PharmD students. Twenty-four students responded (response rate=11%). Most students reported feeling pharmacists should prescribe for minor ailments (92%) and contraception (88%). Students felt least knowledgeable/confident in considering contraindications or warnings, prescribing correct frequency and prescribing correct duration. Students were most concerned with time restraint (22%) and increased workload (20%). They were least concerned with documentation requirements (12%) and knowledge base (7%). In open-ended responses, students expressed a need for further review on the permissible conditions of prescribing, or accessible information for support during the prescribing service. Our study's findings suggest that 4th-year E2P PharmD students agree with prescribing for minor ailments and contraception and generally feel they are comfortable with the knowledge they have currently received on the permissible conditions. However, barriers and a need for further education have been recognized as factors that will deter future pharmacists from implementing prescribing into their pharmacy practice.

INTRODUCTION

The University of British Columbia (UBC) is the sole provider of an accredited Entry-to-Practice Doctor of Pharmacy (E2P PharmD) program in the province of British Columbia (BC) that is designed to prepare graduates for the current and future scope of pharmacy practice (Faculty of Pharmaceutical Sciences, n.d.). The program has its foundation in the educational outcomes and professional competencies outlined by the Association of Faculties of Pharmacy of Canada and strives to train students to be able to assess, optimize, and monitor drug therapy. At the end of their program, students will have achieved these measurable competencies and will use the knowledge and skills they have acquired to practice to their full scope (AFPC, 2018).

The Canadian health care system is facing a shortage of family physicians and other health professionals and as a result, those who are currently practicing feel overburdened with work. In BC alone, there are roughly 1 million residents without a family physician and nearly 40% of those who do have a family doctor are concerned they will lose their health care provider due to practice closure or retirement (British Columbia College of Family Physicians, 2023). Pharmacists in BC are now permitted to adapt prescriptions for a wider range of drugs/conditions and administer a larger number of drugs by injection and intranasal route (College of Pharmacists of British Columbia, 2020; BC Pharmacy Association, 2023). In addition, they now have the authority to prescribe schedule 1 drugs which are medications requiring a prescription and must be sold from licensed pharmacies (College of Pharmacists of British Columbia, 2023; BC Laws, n.d.). These regulations allow pharmacists to prescribe for contraception/emergency contraception and 21 minor ailments (e.g. acne, dysmenorrhea, herpes labialis, shingles and uncomplicated urinary tract infections) (College of Pharmacists of British Columbia, 2023). As pharmacists are one of the most accessible healthcare professionals in the community, the government's strategies are aimed to better utilize their skills and training.

Researchers have investigated student knowledge, confidence, and readiness in prescribing for contraception (Papineau et al., 2021; Lynch et al., 2020; Harris et al., 2020). A descriptive, non-experimental study assessed second year pharmacy students' knowledge and confidence with prescribing hormonal contraceptive products following a simulated case-based activity (Harris et al., 2020). The study found that while students felt confident with prescribing after the activity, there were limitations in their ability to select an appropriate product based on patient-specific factors. Another cross-sectional survey assessed perceived preparedness of providing full-scope pharmacist services among recent PharmD graduates in Ontario (Waite et al., 2018). Investigators measured how able and sure recent graduates felt about services like administering vaccines, conducting medication reviews, and using their independent authority to adapt and prescribe. Overall graduates felt prepared to perform these services, except for less common requests such as prescribing smoking cessation, adapting a dose of a prescription or managing anaphylactic reactions secondary to a vaccine. Factors that decreased their perceived preparedness included younger age of graduates, increased busyness of the pharmacy, and a decrease in the frequency of services performed. The College of Pharmacists of British Columbia have provided a regulatory module for practicing pharmacists to assist them with prescribing (BC Laws, n.d.). However, interventions in the E2P PharmD are required to better equip recent graduates of the program.

METHODS

All E2P PharmD students in their 4th year of the program were invited to take part in an online survey focused on their perceived preparedness to provide minor ailments and contraception services (MACS) (eAppendix). Study invitation and survey link were sent to students on the Canvas platform under the course tab E2P PharmD Program Information Hub, a course available to all students in the program. Fourth year UBC E2P PharmD students registered as student pharmacists with the College of Pharmacists of British Columbia who consented to participate were included. Students in other years of the program and practicing pharmacists were excluded.

The survey questions were based on a comprehensive literature review created by a 4th-year PharmD student (Papineau et al., 2021; Lynch et al., 2020; Harris et al., 2020). The questions were reviewed and refined during a series of meetings by the research team members, all of which are involved in the E2P PharmD program in various roles. Stakeholder feedback was also sought from another 4th-year PharmD student. The questions were designed to evaluate the perceived preparedness of 4th-year PharmD students in providing MACS and to determine what amendments are required within the curriculum to ensure readiness in prescribing. Currently, the UBC E2P PharmD core curriculum contains 5 semesters of medication management which is split into didactic lectures and integration activities (Dahri et al., 2019). Integration activities include various learning approaches where didactic learning is solidified through pharmacy practice lab, case based learning, and small group learning sessions (Dahri et al., 2019).

The survey was available for 1 month with reminder notifications sent 2 weeks after the initial invite and 3 days before the survey closed. The survey was administered on the university's version of Qualtrics, a survey platform that follows privacy regulations. Ethical approval was obtained from the University of British Columbia's Behavioral Research Board (Ethics ID: H23-00859). All participants gave electronic informed consent at the start of the survey and all survey responses were anonymous. Responses were stored in a secure, password-protected database, and all identifiable information (such as names or emails) was anonymized before being provided to the student researcher conducting the data analysis. Students were informed that the survey was not mandatory and their decision to participate would have no effect on their academic standing. Participants were not required to answer all questions in the survey. At the end of the survey, participants had the option to enter into a draw to win 1 of 2 $25 Amazon e-gift cards.

The survey contained 20 questions, 5 assessing baseline demographics, and 15 assessing preparedness to prescribe. Questions varied in style and included Likert, ranking, multiple response, and open-ended formats. All question responses were analyzed using Excel version 16.75 (Microsoft Corporation). Descriptive statistics was done using proportions and percentages for all Likert and multiple response type questions. For the questions that allowed students to select multiple responses, data was analyzed based on total number of responses to the question instead of overall number of survey respondents. The ranking question was analyzed using a ranking system approach (Lee et al., 2023). For example, if a participant was to rank an item to be most knowledgeable (selected as #1), a score of 1 was assigned to the item. Subsequent items were scored up to 9. A cumulative score was summed from all participants for each individual item and the items were ranked overall from most knowledgeable (having the lowest number of points) to the least knowledgeable (having the greatest number of points). Open-ended responses were analyzed using thematic analysis, where responses were grouped based on common ideas and subject matter to summarize the results and find shared opinions between student responses. Each open-ended question was analyzed independently, and some responses were added in more than one category if they addressed more than one theme. Final categorization was discussed with key stakeholders and feedback was incorporated to refine the analysis and confirm the categorization approach.

RESULTS

All 4th-year E2P PharmD students (N=212) were invited to participate in the survey. A total of 40 students participated in the preliminary questionnaire 30 of whom met the inclusion criteria. Of the eligible students, 26 completed the 5 questions regarding baseline demographics, but 2 students did not continue the survey following the fifth question. Thus, a total of 24 students meeting the inclusion criteria completed the survey (response rate= 11%). Table 1 summarizes participants' baseline demographics in terms of experiential education practicums, employment status after graduation, and references used. The majority of participants

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agreed that pharmacists should prescribe for minor ailments (92%) and contraception (88%). Although none of the participants disagreed, some felt undecided about prescribing for minor ailments (8%) and contraception (12%). When asked about pharmacists prescribing all medications, only about half (54%) agreed to the statement while the other half either disagreed (8%), felt undecided (29%) or selected other (8%). Students that selected 'other' provided comments in a response field, including a need for additional training, concerns about the time required to fully assess patients, and the suggestion to limit prescribing authority to pharmacists with a Doctor of Pharmacy degree and hospital pharmacists.

Table 1. **Data on participants' experiential education practicums, employment status after graduation, and references used are presented.** For experiential education, percentages were calculated based on the number of respondents (n=26) for completion rates and specialty area involvement, and on the total number of responses (n=30) for practicum settings (multiple responses allowed). Employment status was calculated based on the number of respondents (n=26), including both overall employment and employment in British Columbia. For references used, percentages were calculated from a total of 100 responses (n=100). Participants were also given the option to provide additional references, which included: Louisville Kidney Disease Program, Bugs and Drugs, RxVigilance, MedSask, Firstline, Natural Medicines, Guidelines/lectures, BC Renal, CCS, CADDRA, Primary/Secondary Literature, and Pyrls.

Category	*Subcategory*	*Number (%)*
Experimental Education Practicums	Completed all experimental education practicums (n=26)	14 (54)
	Any experimental education practicums in a specialty area (n=26)	18 (69)
	PHRM473 practicum in an outpatient setting (n=30)	16 (53)
	PHRM473 practicum in an inpatient setting (n=30)	5 (17)
	PHRM473 practicum in non-direct patient care setting (n=30)	9 (30)
Employment after Graduation (N=26)	Total students who have confirmed employment after graduation	22 (85)
	Students who have confirmed employment after graduation in BC	18 (69)
References used to Assist in Medication Management (N=100)	Lexicomp	26 (26)
	eCPS	25 (25)
	RxFiles	21 (21)
	UpToDate	19 (19)
	Other	9 (9)

Students were asked to express opinions using a 5-point Likert scale ranging from strongly agree to strongly disagree. A series of 5 questions were asked to assess comfort in various aspects of prescribing. Most students fell into the agree and strongly agree categories (Figure 1). Of the questions asked, students felt most comfortable with selecting a therapeutic regimen for minor ailments and least comfortable with assessing and selecting a therapeutic regimen for contraception. Students' ranking of recommending drug therapy from most to least knowledgeable and confident is summarized in Table 2. Students felt most knowledgeable and confident in assessing safety based on allergies and felt least knowledgeable and confident in prescribing the correct duration.

Table 2. **Aspects of recommending drug therapy.** Ranked with #1 being most knowledgeable/ confident and #9 being least knowledgeable/confident.

Ranking	*Aspects of Recommending Drug Therapy (n=23)*
1	Assessing safety based on allergies
2	Assessing presenting symptoms
3	Following current clinical guidelines
4	Assessing drug interactions
5	Considering patient preferences
6	Prescribing the correct drug and strength
7	Considering contraindications or warnings
8	Prescribing the correct frequency
9	Prescribing the correct duration

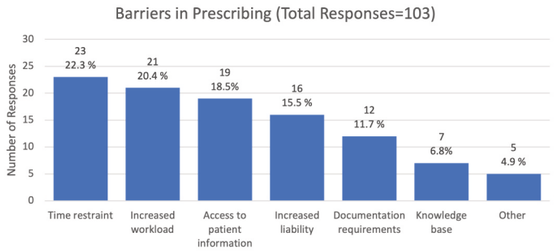

Figure 2. **Factors influencing participants' ability to prescribe.** Percentages calculated based on number of responses.

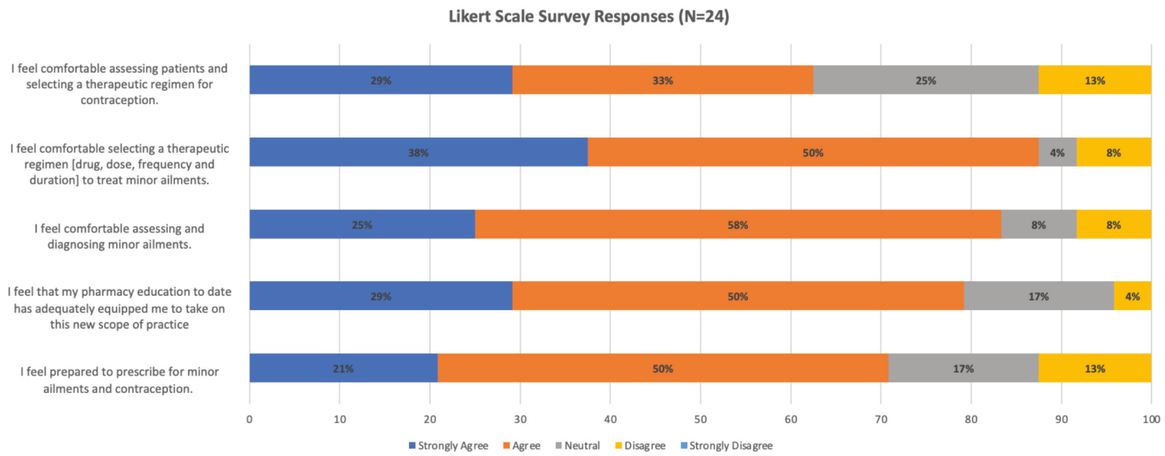

Figure 1. Likert Scale Responses on aspects of MACS prescribing.

Students were asked to select potential barriers that may impact the ability to prescribe (Figure 2). Students were most concerned with time restraint (22%) and increased workload (20%) and least concerned with knowledge base (7%). When asked if the students would prescribe in the coming months after graduations, 58% replied with yes, 13% did not think they would prescribe, and 29% were still undecided. Many students (83%) believed pharmacists' role in delivering care in the community would change.

Students were asked to describe their overall feelings with respect to the implementation of prescribing authority in BC (27 responses). The overarching opinion was of ambivalence (15 responses); they felt competent, but expressed concerns regarding workload, burnout, being understaffed, increased liability, lack of guidance in prescribing from UBC, and overall nervousness. Other students (5 responses) expressed their excitement of practicing to their full scope. A total of 2 students stated being nervous with prescribing. A few students (5 responses) felt capable to prescribe, however expressed compensation concerns.

Students were asked what additional educational support would be needed to help prepare them for MACS (n=22). Nearly half the students (10 responses) stated needing extra education or training specific to the list of permissible conditions. Others (7 responses) recommended guidelines and accessible information that would help in efficiency and efficacy of prescribing. Similarly, 3 responses recommended introducing pre-printed orders to improve consistency between pharmacists' assessments. Two responses stated needing no additional educational support.

Students were asked if they have any other thoughts on pharmacists prescribing authority in BC (n=13) to which responses were varied. Some students (2 responses) raised concerns regarding increased workload and burnout. Others (3 responses) expressed some level of nervousness regarding the new regulations and implementing these in their first year of practice. The topic of compensation (4 responses) was reiterated. Two students addressed the limited list of conditions and the potential to expand authorization to prescribe all medications, except narcotics and controlled substances. One student expressed curiosity towards how MACS would look in the hospital setting. The remaining 10 students did not raise any further concerns.

DISCUSSION

The objective of the study was to understand the perceived preparedness of 4th-year E2P PharmD students in providing MACS. Fourth-year E2P PharmD students generally agreed with the new regulation changes, but not all were sure they would implement it when out in practice. While participants generally felt prepared and comfortable in prescribing for MACS, they did identify concerns around prescribing.

As expected, the majority of students were in agreement with the new regulations for MACS; however, not all students were sure they would implement it when out in practice. Although students felt comfortable selecting a therapeutic regimen, when asked to rank their knowledge and confidence, prescribing correct frequency and duration were ranked the lowest. Expectedly, assessing based on allergies, assessing presenting symptoms, and following clinical guidelines were ranked high in knowledge and confidence, as these concepts are taught throughout the program. Key barriers that students felt would impact their ability to prescribe were time restraints and increased workload. While the knowledge base was ranked as the least significant barrier, students still reported a need for additional education and training on the new regulations. These results suggest that although students may feel their general knowledge base is adequate, they require further knowledge that is specific to diagnosing and prescribing.

We found that participants were least comfortable with assessing and selecting a therapeutic regimen for contraception. As there are a variety of different forms of contraception, lack in comfort may be due to providing the correct contraception product based on patient specific factors, such as preference of dosage form, desired time to return to fertility, and adherence concerns (Harris et al., 2020). Similarly, a study evaluating American pharmacy students' ability to prescribe contraception found that students were most confident in gathering information and least confident in recommending the correct contraceptive (Lynch et al., 2020). Students then took part in a simulated lab activity, after which they reported an increased level of confidence in their ability to prescribe the contraceptive service. Didactic learning of a minimum of 4-6 hours was shown to provide increased level of confidence in contraceptive prescribing (Lynch et al., 2020). Students in the E2P PharmD program may benefit from targeted training specific to MACS prescribing during integration activity sessions in combination with didactic learning within the program.

Students identified assessing allergies and presenting symptoms as the areas they felt most knowledgeable and confident in when recommending drug therapy. This result aligns with what is taught in different aspects within the E2P PharmD curricula, which includes experiential education practicums (Lau et al., 2019; Mira, 2019). This finding is similar to previous research of 4th-year pharmacy and medical students in Alberta where pharmacy students felt most confident in assessing safety based on allergies (Woit et al., 2020). In this same study, it was found that pharmacy students were least confident in prescribing the correct duration and considering contraindications or warnings. These findings are consistent with our study as the three lowest ranked concepts of recommending drug therapy were: contraindications or warnings, prescribing correct frequency and prescribing correct duration. These results highlight potential gaps in training, particularly in areas of more complex decision-making. Students with limited hands-on experience in real patient cases may feel less confident in their ability to make informed decisions.

Barriers identified in our survey were possible increased workload and time restraints. Similar concerns have been found in a study of recent graduates of a PharmD program in Ontario (Waite et al., 2018). Although new graduates felt confident in their ability to provide pharmacy services, for every 1-unit increase in busyness (rated 1–6, with 1 being slow and 6 being frantic), their confidence in practicing to their full scope decreased. This study was conducted prior to the new regulations introduced in January of 2023 in Ontario which includes prescribing for 13 minor ailments

(Bronstein, 2023). As scope of practice increases, the busyness of pharmacies would also increase, possibly causing further challenges in practicing the full scope decreased (Waite et al., 2018). Time constraints, increased workload, and lack of prescribing-specific education may make it difficult for newly graduated pharmacists to implement this essential service in their practice.

Concerns were also identified around compensation for prescribing, a barrier also found in previous literature (Zhou et al., 2019; Pharmacy Practice Research Abstracts, 2019). Insufficient compensation could be a potential downfall to prescribing as the newly graduated pharmacists may not be inclined to prescribe due to the increased responsibility and low return in direct compensation.

In a mixed methods study, Alberta pharmacy students and pharmacists were interviewed and surveyed to understand what additional educational needs were required with the expanded scope of practice (Schindel et al., 2019). These students expressed a preference for experiential rather than didactic learning. In contrast, our study found the students expressed a need for additional education in the form of lectures or refresher courses. A combination of experiential and didactic learning would increase students' confidence in prescribing (Lynch et al., 2020). If students have been exposed to the process of prescribing in school, they will be inclined to implement it into practice as licensed pharmacists. It would also be beneficial to add prescribing for MACS into experiential education practicums as students would be practicing these skills in the workplace. In this case, students will experience realistic situations with their preceptor's guidance while didactic learning and integration activities will solidify their knowledge in prescribing for MACS.

There were a number of limitations in our study. The response rate was lower than expected due to the timing of the survey dissemination, limiting the generalizability of the results. The mode of the invitation to participate, being posted on a general course module learning system site rather than a course specific site, could also have impacted our dissemination efforts. Additionally, the low response rate raises the potential for non-response bias, as respondents may differ from non-respondents in ways that could influence the results. For example, those who chose to participate may have had stronger opinions or experiences related to the topic. Lastly, as we did not enforce required answering fields, there was variability in the students' response rates to different questions, which resulted in different cohort size for each answer.

CONCLUSIONS

In conclusion, 4th-year E2P PharmD students have expressed their agreement in providing MACS and generally feel they are comfortable with the knowledge they have currently received on the MACS conditions. However, barriers and a need for further education has been recognized as these factors may deter future pharmacists from implementing prescribing into their pharmacy practice. Further qualitative research may be needed to provide insight on which teaching methods are effective in preparing PharmD students with providing MACS.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Lake primary producer community responses to anthropogenic use

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ABSTRACT Anthropogenic activity around a lake is usually accompanied by eutrophication of its waters. While the relation between eutrophication and dissolved nutrients is established, direct relationships between anthropogenic activity and biotic variables related to lake eutrophication are less well studied, especially in early stages. We evaluated the effect of what is still a relatively light human presence on primary producer communities in lac Lusignan, a north temperate lake (Quebec, Canada). Human recreational activities around the lake have increased in recent years, primarily towards the lake outflow. To account for varying degrees of human activity and vegetation type along the lake's shoreline, we classified sites into four types: human housing (Inhabited) and forest (East-forest) on the eastern bank and peat bogs (Bog) or forests (West-forest) along the western bank. We measured dissolved nutrients (nitrates and phosphorus) in the water column, as well as four primary producer responses: cyanobacterial density, littoral periphyton thickness, and macrophyte biomass and diversity. We expected that more intensively used areas of the lake (Inhabited East bank sites) would have thicker periphyton, higher macrophyte biomass and diversity, and greater cyanobacteria density; all characteristics related to eutrophication and anthropogenic influence. Nitrate was higher along the western shore sites and phosphorus concentrations tended to be higher in Bog and Inhabited areas. Periphyton was thickest in Inhabited sites, while macrophyte biomass and diversity were reduced. Cyanobacterial communities were particularly dominated by the potentially toxic and nuisance genus *Dolichospermum* in Inhabited sites. Our study demonstrates symptoms of eutrophication in this historically unperturbed lake and indicates that attention should be paid to regulating the densification and spread of human activity to preserve water quality.

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INTRODUCTION

In most north temperate lakes, nutrients (especially phosphorus) limit the growth of primary producers such as macrophytes, littoral periphyton (microalgae growing on underwater surfaces) and cyanobacteria. Enrichment with nutrients, primarily phosphorus -a process referred to as eutrophication- favours organisms that can fix atmospheric nitrogen (N), which is the next limiting nutrient in lakes (Barroin, 2003). The primary aquatic N-fixers are cyanobacteria (Downing et al.,1997; Zhang et al., 2023), and their proliferation, in addition to being potentially toxic for other organisms, results in reduced water transparency and dissolved oxygen (O'Neil et al., 2012). Lake eutrophication is often associated with human activity in the watershed, which increases nutrient input either directly (e.g., leaky septic systems) or through runoff (e.g., lawn fertilizers). Eutrophication can be difficult to reverse (Beisner et al., 2003) and may have significant effects on the diversity and functioning of the ecosystem, leading to its degradation (Dillon & Molot, 2024). In addition to human activities, some natural environments, such as bogs, can be natural nutrient sources owing to high decomposition rates and intermittent drying periods (Wright et al., 2009). While eutrophication is often studied in lakes that are already enriched, it is less common to examine the early indicators of this phenomenon.

With climate change, more northern regions in Canada are increasingly inhabited or used recreationally. In the province of Québec, lac Lusignan (Fig. 1) has historically had little human residential or recreational activity as it is located on public lands but is managed privately for fishing and hunting. Over the past decade, about a dozen new permanent dwellings (cottages) have been constructed in the area, and pleasure craft and campground usage have increased. It should be noted that all these activities are relatively limited given that this is still a protected area for wildlife. To this point, all the properties, which are limited to the eastern bank of the lake, are accessed by an unpaved dirt road; this is not yet a site of major urban development. The western shore, with no road access, is relatively untouched. Our goal was to determine whether there is a detectable signal of these small but notable increases in anthropogenic pressure on the lake. Given that they are generally affected first by eutrophication, our study focuses on the major primary producers in lakes: periphyton, macrophytes, and cyanobacteria, as well as nutrient concentrations in the water.

Generally, we expected that increases in nutrients would result from greater human activity. However, primary producers can quickly remove nutrients from the water column unless lakes are already highly eutrophic. Thus, in early stages of eutrophication, primary producer biomass could be more indicative of a trend towards eutrophication than would dissolve nutrient concentrations alone. Periphyton are agglomerations of bacteria, algae and protozoa that grow as crusts on submerged solid surfaces in the littoral zone of lakes (Vadeboncoeur & Steinman, 2002) and are indicators of nutrient enrichment (Hill & Fanta., 2008, Fanta et al., 2010, Porter-Goff et al., 2010). We hypothesized that a thicker periphyton cover would occur where human activity was greatest. Macrophyte biomass and diversity have also been used as bioindicators in lakes, for monitoring the impact of anthropogenic activities (Camargo, 2018). Based on previous studies (Islam, 2009; Akasaka et al, 2010), we hypothesized that macrophyte diversity and biomass would be reduced in Inhabited sites compared to the forested sites because humans will often remove macrophytes to enable more pleasant swimming and boating. Finally, because anthropogenic activity can lead to nutrient enrichment that aids the proliferation of harmful and nuisance cyanobacterial species such as *Microcystis*, *Dolichospermum*, *Aphanizomenon* and *Aphanocapsa* (Perri et al., 2015, Zhang et al., 2023), we hypothesised that these taxa would be more common in Inhabited sites.

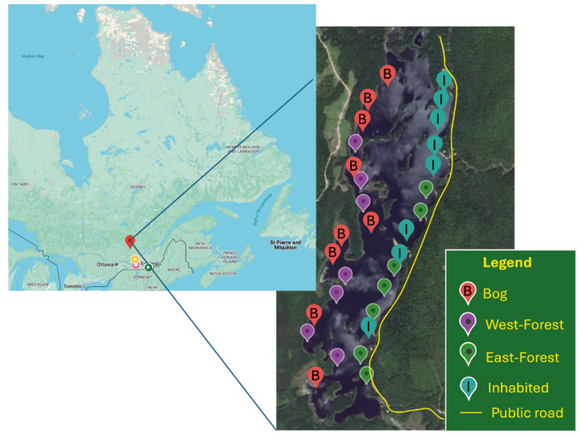

Figure 1. Map of lac Lusignan with inset showing its location in the province of Québec. The 4 km length of the lake was divided into 15 segments of 267 m each. The Inhabited site sampling locations are indicated with points coloured in green, the East-forest zones are in turquoise, the Bog sites in red and the West-forest sites in purple.

MATERIALS AND METHODS

Water flow in lac Lusignan (46o 41' 08" N, 74o 08' 51" W, Saint-Michel-des-Saints, Quebec, Canada; Fig. 1) is along the main axis from the northern to the southern boundary where a dam maintains higher water levels. The east and west lakeshores define the main axis (fetch) of the 4 km long main lake basin (relative to a mean 1 km width). Samples were collected at 15 sites per shoreline spaced 267 m apart (to divide the total lake length equally). Sites were then assigned to one of four major shoreline site types based on land-cover or use, for statistical analyses. The eastern shoreline consisted of (i) forest-dominated sites (East-forest) and (ii) human Inhabited sites, while the western shoreline consisted of (iii) the forested West-Forest interspersed with (iv) peat bogs (Bog). Bog sites were composed mainly of wet soils, with a high level of decomposing plant biomass, and thus could represent nutrient-rich areas (Wang et al., 2015). The East-forest was interspersed by a few isolated dwellings (classified as Inhabited), and the West-forest was interspersed by Bog sites. We compared nutrient concentrations and primary producer indicators by site types.

All sampling was done between September 5 and 9, 2023, with data for each variable collected at all sites within the same day. To assess water column nutrient concentrations, we collected samples at 1 m depth at each site (100 m from the shore) using a Van Dorn bottle and returned the water to the lab in dark Nalgene bottles, which were refrigerated until analyzed within a 24-hour period. We measured total phosphorus (TP) and nitrate (NO3, one of the most abundant and biologically usable forms of nitrogen) in two different subsamples of unfiltered water. Total phosphorus was assayed on a spectrophotometer (GE Ultrospec 2100 pro, Piscataway, NJ, USA) at 890 nm (Murphy and Riley, 1962). Nitrate was measured colourimetrically using the same spectrophotometer at 543 nm (Morris and Riley, 1963) with the ammonia measurement protocol developed by Solarzano (1969).

We measured periphyton thickness directly at each site using a ruler. Whenever possible, periphyton was measured on two pieces of wood (submerged sticks), or where not available, on rocks. Macrophyte collections were done with duplicate quadrats taken at all fifteen sites. At a water level of approximately 50 cm, all above-ground biomass in a 50 cm x 50 cm randomly placed quadrat was collected by cutting plants at the sediment surface. Plants were dried in an oven at 80℃ for 48 hours and then weighed to the nearest gram. To quantify macrophyte species richness, we identified and counted on site, all plants within duplicate randomly placed 50 cm x 50 cm frames at the same lakeshore sites. Where field identification was not possible, samples were brought back and identified in the laboratory. From these data, we estimated diversity using the Shannon index, H (Shannon, 1948).

Cyanobacteria were sampled in the epilimnion (surface) of the lake, where cyanobacteria density is highest (Wilkinson et al., 2020), at the same time and sites as the nutrient samples. Cyanobacteria were collected using a 30 µm mesh net haul from 2 m depth to the surface. Samples were washed into glass bottles and preserved with Lugol's solution. In the lab, 45 mL samples were centrifuged (15 minutes at 3000 rpm) to concentrate the cyanobacteria, which were then analyzed microscopically to quantify the density of each genus.

We compared the response variables (TP and NO^3 concentrations, periphyton thickness, macrophyte biomass and diversity (H), total cyanobacterial density and genus densities) across the four site types (Inhabited, Bog, East-Forest and West-Forest) using ANOVA

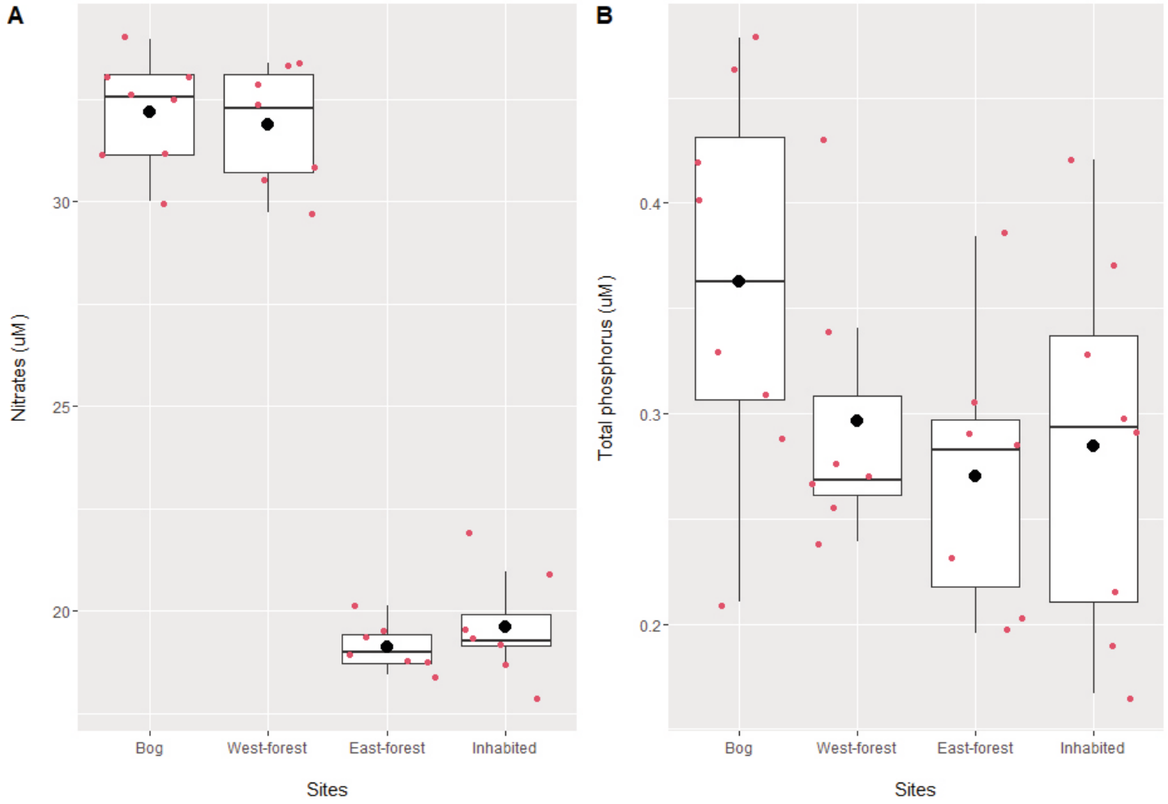

Figure 2. Boxplots showing median values (mid-box lines), means (black dots), data distributions (red dots) and standard deviations (whiskers) for (A) nitrate and (B) total phosphorus concentrations (untransformed) for the four site types.

Table 1. ANOVA and post hoc Tukey results for abiotic and biotic indicators of eutrophication between Bog (BG), West-forest (WF), East-forest (EF) and Inhabited sites. Significant differences ($p < 0.05$) are indicated in bold. For macrophyte diversity, the Welch test with Games-Howell post hoc test results are also shown. Asterisks indicate non-significant tendencies ($p < 0.1$).

	ANOVA			POST HOC TEST RESULTS					
	df	F	P	BG-WF	BG-IH	BG-EF	WF-IH	WF-EF	IH-EF
A) Abiotic									
Nitrates	3	274.14	**<0.001**	0.951	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.870
Phosphorus (log)	3	1.76	0.79	0.539	0.255	0.195	0.963	0.904	0.996
B) Biotic									
Periphyton thickness	3	8.44	**<0.001**	0.449	**<0.001**	**0.044**	**0.018**	0.598	0.253
Macrophytes:									
• Biomass (log)	3	2.36	**0.018**	0.704	**0.046**	**0.031**	0.390	0.285	0.993
• Diversity	3	4.73	**0.018**	**0.026**	0.763	0.263	0.066*	0.649	0.224
Cyanobacteria density	3	3.31	**0.036**	0.332	0.843	0.306	0.080*	1.000	0.072*
C) Genera									
Dolichospermum	3	2.60	0.074*	0.560	0.567	0.830	0.071*	0.968	0.176

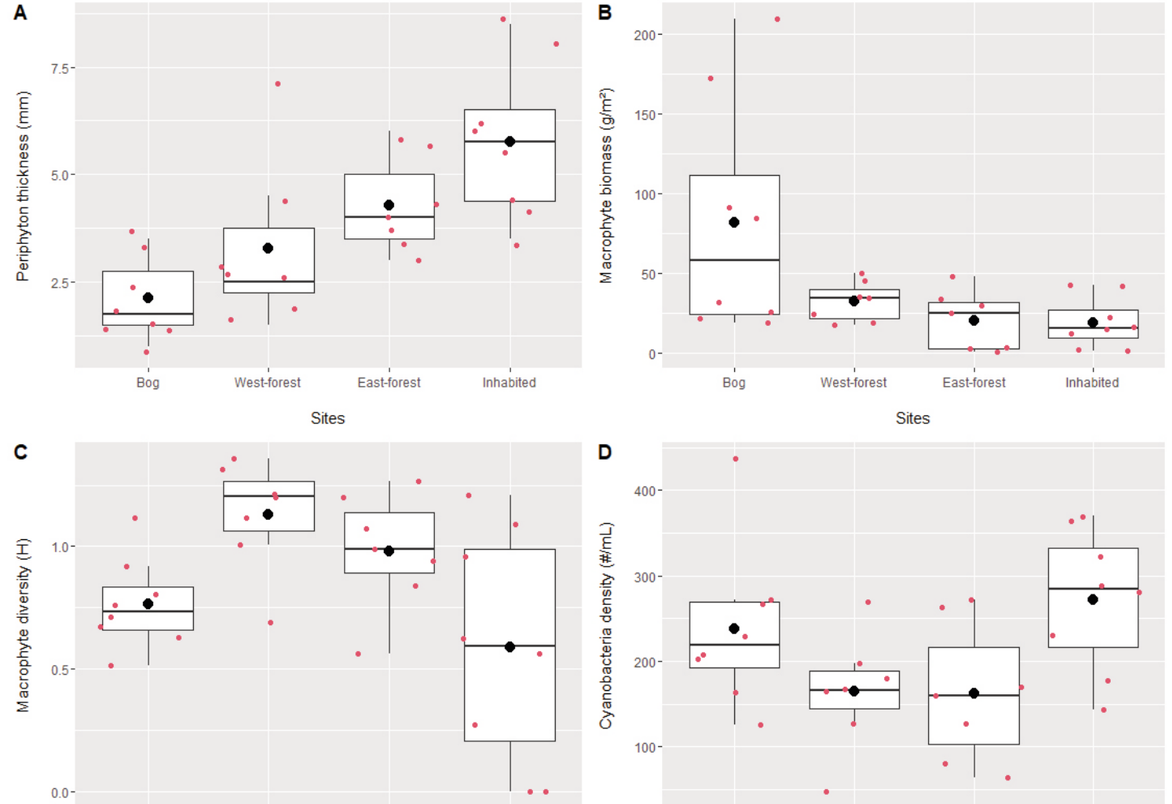

Figure 3. Boxplots of untransformed (A) periphyton thickness, (B) macrophyte biomass, (C) macrophyte diversity (Shannon index) and (D) Combined cyanobacteria density for the four site types. Shown are the median values (mid-box lines), means (black dots), data distributions (red dots) and standard deviations (whiskers).

followed by a Tukey HSD when data were normally distributed and homoscedastic. The assumptions of normality were tested through visual assessment of diagnostic graphs, while assumptions of homogeneity of the variance were tested using the Levene's test. Response variables for total phosphorus, macrophyte biomass and site densities of two cyanobacteria genera (*Gomphosphaeria* and *Woronichinia*) were log10-transformed to achieve homoscedasticity and also normality in the case of macrophyte biomass. Data were also not homoscedastic for macrophyte diversity and overall densities of cyanobacteria genera. Because data transformation could not remedy this situation, Welch ANOVA tests followed by Games-Howell post hoc tests were used instead of the ANOVA and Tukey tests. All analyses were done in R version 4.2.2 (R Core Team) using R-Studio build 2022.12.

RESULTS

Nutrients

Nitrate concentrations were significantly different between site types ($F_{(1,3)} = 274.14$, $p < 0.001$) (Figure 2A; Table 1A). Both the Bog and West-forest sites had higher nitrate concentrations than the Inhabited and East-forest sites ($p < 0.001$; Table 1A), indicating differences that were more associated with the side of the lake rather than the site-specific land use type. For TP, we found no significant differences between the sites ($F_{(1,3)} = 1.76$, $p = 0.179$) (Figure 2B; Table 1A); however, Bog sites tended to have higher phosphorus concentrations than the three other site types (Table 2A).

Periphyton

Periphyton thickness differed significantly between the site types ($F_{(1,3)} = 8.44$, $p < 0.001$), being significantly thicker in Inhabited sites than in Bog ($p < 0.001$) and West-forest sites ($p = 0.018$) (Figure 3A; Table 1B). Inhabited areas also tended to have greater periphyton thickness than East-forest areas (Table 2B); however, this difference was not significant ($p = 0.253$). Periphyton thickness in Bog sites was lower than in the forested sites along the eastern shore ($p = 0.044$).

Macrophytes

Macrophyte biomass differed significantly between sites ($F_{(1,3)} = 2.36$, $p = 0.018$) (Figure 3B; Table 1B), being greater in Bog sites than in Inhabited ($p = 0.046$) and East-forest sites ($p = 0.031$; Table 2B). Macrophyte diversity also differed significantly between site types (Welch $F_{(1,3)} = 4.73$, $p = 0.018$) (Figure 3C, Table 1B). Macrophyte diversity tended to be lower in Inhabited sites compared to the West-forest, but this was not significant at the $\alpha =$

Table 2. Means and standard deviations (untransformed values) for the response variables by site type.

	A) Abiotic				B) Biotic								C) Genus	
	Nitrates (µm)		Phosphorus (µm)		Periphyton (µm)		Macrophyte Biomass (g/m²)		Macrophyte Diversity (H)		Total Cyanobacteria (n/mL)		Dolichospermum (n/mL)	
Site Type	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bog	32.20	1.31	0.363	0.09	2.125	0.95	82.14	73.35	0.766	0.19	238.3	93.76	101.5	48.40
West-forest	31.87	1.48	0.296	0.07	3.286	1.89	32.60	12.53	1.128	0.23	164.6	67.76	62.86	50.16
East-forest	19.12	0.59	0.270	0.07	4.286	1.11	20.74	18.43	0.982	0.24	162.3	81.85	76.57	46.24
Inhabited	19.60	1.25	0.284	$^{0.09}$	5.750	1.79	19.41	15.77	*0.589*	*0.47*	*272.3*	*82.73*	138.5	74.77

0.05 level in the Games-Howell post hoc test (p = 0.066). Moreover, macrophyte diversity was significantly lower in Bog sites compared to West-forest (p = 0.026) (Table 1B). Generally, both Bog and Inhabited sites had lower mean macrophyte diversity than the two forested sites (Table 2B).

Cyanobacteria responses

Total cyanobacteria densities summed across all genera differed significantly between site types (F (1,3) = 3.31, p = 0.036) (Figure 3D). Post hoc tests only showed non-significant trends, however. Total densities tended to be greater in Inhabited sites than in the two forested site types (p = 0.072 for East-Forest and p = 0.080 for West-Forest), while Bog sites were intermediate in density (Table 2B).

A Welch ANOVA was used to compare and contrast the densities of the nine genera of cyanobacteria observed independent of site type (Figure 4), and significant differences were observed (F(1,8) = 33.65, p < 0.001; Table 3). *Dolichospermum* and *Aphanocapasa* had significantly greater densities than all other cyanobacterial genera (all p < 0.001) (Figure 4; Table 3 post hoc group A) followed by *Gomphosphaeria* with higher densities than seven other genera (all p < 0.001, except p = 0.010 with *Aphanizomenon*) (Figure 4; Table 3 post hoc group B). Finally, *Aphanothece* (Table 3 post hoc group C) was significantly denser than *Aphanizomenon* (p < 0.001), *Chroococcus* (p = 0.002), *Microcystis* (p < 0.001) and *Woronichinia* (p < 0.001) (Figure 4; Table 3 post hoc group D).

We found no significant density differences between sites at α = 0.05 for any taxa. *Dolichospermum* tended to have higher mean densities in Inhabited sites, where mean densities were almost double over both forested types (Table 2C), compared to other site types, especially the West-forest (p = 0.071) (Figure 4 inset). Notably, *Microcystis* was observed only along the eastern shore in the Inhabited and East-forest sites; this species was completely absent from the uninhabited western shore.

DISCUSSION

We explored a variety of indicators of human activity impacts on the water quality (nutrients) and primary producers in lac Lusignan, a lake that has experienced increased anthropogenic impact in recent years, although it still has an overall low human use with only unpaved, dirt road access. Contrary to our expectation that greater human activity on the Eastern shore would lead to higher nutrient concentrations, nitrates were higher in the uninhabited Western shore sites and TP did not vary significantly between shores or sites. Thus, differences in only nitrates occurred, and these were linked more to shoreline rather than to local site land-use, indicating little measurable nitrate input by humans at this time. For phosphorus, however, non-significant trends were present, which partially support our hypotheses. Bogs tended to have higher TP than other site types, as might be expected given that they are generally areas of high plant productivity and subsequent decomposition (Sottocornola et al., 2007). Human-inhabited areas tended to have the next highest TP, concurring with our initial expectations of anthropogenic eutrophication. It is possible that the interspersion of the different site types along the lakeshores could explain the lack of significant differences. With a more intensive sampling through time or considering only sites surrounded by the same land-use/cover type, some of the observed trends may become significantly different. It should be noted that the upper range of TP observed (~0.7µm) corresponds to a lower-mesotrophic status according to the Québec Ministry of Environment classification scheme (MELCCFP, 2024), with most of the TP values within the oligotrophic range; this supports the contention that this lake has not become severely mesotrophic or eutrophic yet.

Given its primarily oligotrophic status, it is possible that any extra dissolved nutrients entering the lake through human activity have already been converted into greater primary producer biomass (Hansson, 1990; Vadeboncoeur & Lowe, 2024), rendering primary producers more useful indicators of early eutrophication. In particular, periphyton, which had thicker mats in Inhabited sites, could have reduced nutrient concentrations in the water at these sites. We also observed a periphyton response that was, overall, opposite to that of the macrophytes; a finding that is not surprising given that macrophytes often occupy the same littoral habitat as periphyton and likely compete with them. High macrophyte biomass on the western shore and in Bog sites likely increased competition for nutrients and light availability, thereby limiting periphyton growth in those areas (Sand-Jensen & Borum, 1991; Hill & Fanta, 2008). Conversely, active removal of macrophytes by people to enable more pleasant swimming and boating can favour periphyton growth at Inhabited sites. Such plant removals improve nutrient and light access for the benthic periphyton associated with rocks and woody debris, while negatively impacting macrophytes (Lambert et al., 2008; Porter-Goff et al., 2010).

These observations also provided support for our hypothesis that macrophyte biomass would be reduced in Inhabited areas and be highest in Bog sites, which was generally upheld. As mentioned, for the Inhabited sites, our finding suggests that human activity negatively impacted macrophyte biomass and diversity in the lake, presumably from active removals for beach and dock development, thereby reducing vascular aquatic plant biomass

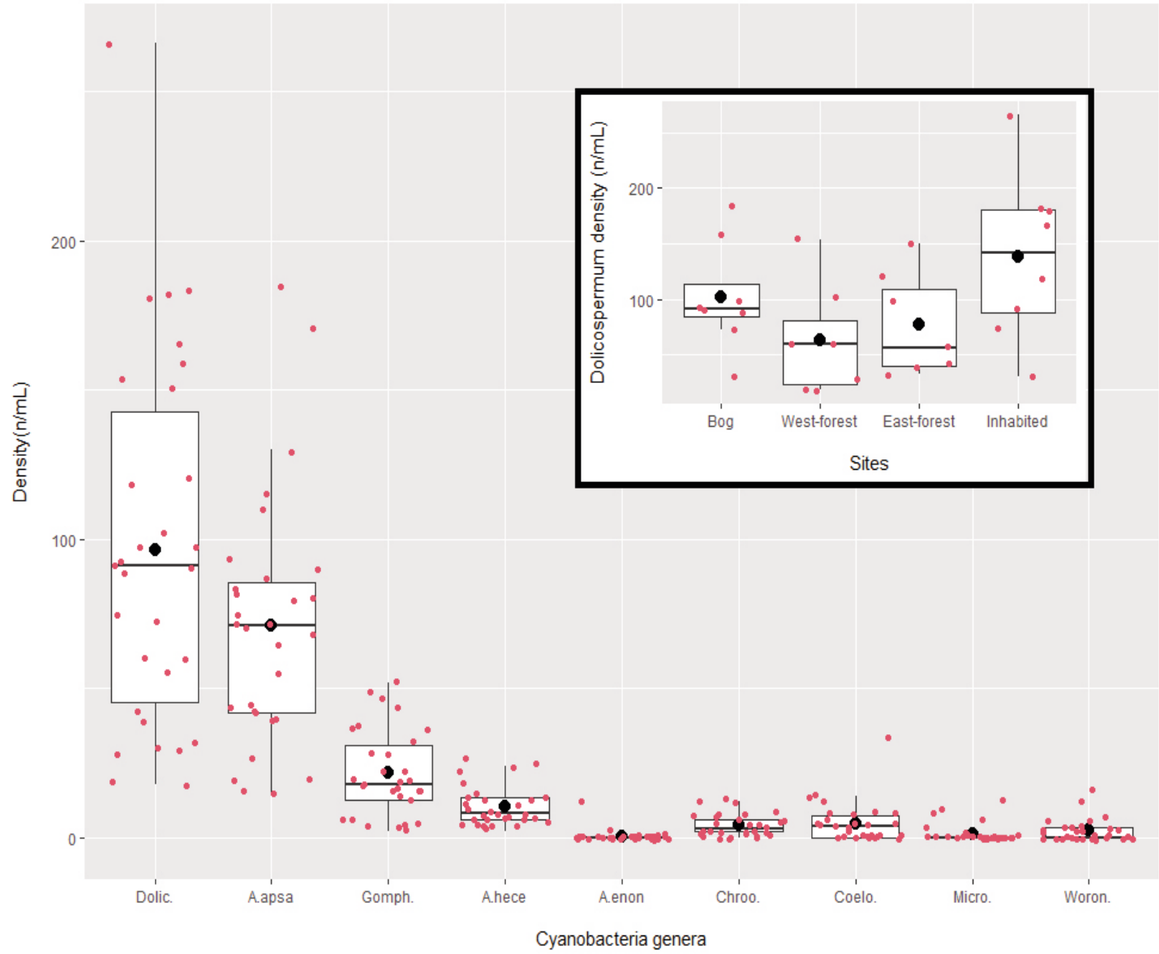

Figure 4. Boxplots for cyanobacteria genera untransformed densities. Nine genera were identified: *Aphanocapsa* (A.apsa), *Aphanizomenon* (A.enon), *Aphanothece* (A.hece), *Chroococcus* (Chroo.), *Coelosphaerium* (Coelo.), *Dolichospermum* (Dolic.), *Gomphosphaeria* (Gomph.), *Microcystis* (Micro.) and *Woronichinia* (Woron.). Inset: Trends (non-significant with p = 0.07) in *Dolichospermum* densities by site type.

Table 3. Mean densities and standard deviations for each cyanobacteria genus, using untransformed values, averaged across all sites. Differences among site types were estimated by ANOVA with p-values shown. Differences in densities (across all sites) were determined using a Welch ANOVA followed by a Games-Howell post hoc test. Shared letters in the post hoc column indicate no significant differences between genera. The NA for *Aphanizomenon* resulted from its rarity across sites (many 0's and extremely low densities when found).

	Density (n/mL)		Site ANOVA	Welch ANOVA	Post hoc genus
Genus	Mean	SD	*p*-value	test	group
Dolichospermum	96.533	61.161	0.074	$F(1,8) = 33.648$	A
Aphanocapsa	70.933	41.558	0.164	$p \leq 0.001$	A
Gomphosphaeria (log)	21.533	14.410	0.154		B
Aphanothece	10.267	6.659	0.913		C
Aphanizomenon	0.533	2.224	NA		D
Chroococcus	4.133	3.785	0.948		D
Coelosphaerium	4.733	7.017	0.900		D
Microcystis	1.400	3.244	0.129		D
Woronichina (log)	2.333	$3.^{790}$	0.336		D

(Thiemer et al., 2021). On the other hand, the Bog sites were nutrient-rich environments that had high associated macrophyte biomass, but a low to intermediate diversity.

For cyanobacteria, we hypothesised that increasing anthropogenic presence would result in measurable increases in cyanobacterial densities. Overall density did tend to be higher in the Inhabited sites—sites that were associated or expected to be associated with higher nutrient inputs, especially TP, which is most important for cyanobacterial proliferation (Perri et al., 2015). The increases were relative to the forested sites. Within the cyanobacteria, the potentially toxic *Dolichospermum* was found in high densities across our sites, being almost double in the Inhabited sites compared to the forested sites, in line with our hypotheses. The growth of this taxon is influenced primarily by excessive nutrient discharges from anthropogenic sources into watercourses (Perri et al., 2015). *Aphanocapsa* was also found at high densities, being assigned to the same post hoc group. Unlike many nuisance cyanobacteria like *Dolichospermum*, *Aphanocapsa* is a non-nitrogen-fixing colonial picoplankton that can be found in sites ranging from relatively oligotrophic (Padisak et al. 2009; Watson et al., 2017) through to eutrophic (but not hypereutrophic) (Kuczyńska-Kippen et al. 2024). Interestingly, *Microcystis*, a common toxin and bloom-forming taxon, was only observed on the human-occupied eastern lake shoreline, pointing to effects of human activity here as well.

Studies such as ours are entirely correlative in nature and cannot directly point to a causal relationship between human activity and the changes we detected in primary producers. Other caveats include having a team of students working to identify the cyanobacteria taxa which could introduce observer error. We worked to minimize this by having identifications being done simultaneously and with double-checking by other team members. Another limitation of our study is that we were only able to observe the lake at a single time-point. Having a more dynamical perspective over seasonal, but also inter-annual time scales would be a useful extension of our work. Overall, our preliminary study suggests that human activity, influencing the East bank of lac Lusignan, has a measurable correlation with some aspects of dissolved nutrients and primary producer communities. The relations we observed were generally small, which is not surprising given that the lake has only recently become subject to increased anthropogenic activity and is likely in the early stages of eutrophication, having only reached low mesotrophic status at the highest measured TP levels. It is thus probably not too late to put into place interventions that could reverse this trend. As climate change warms the waters of more northerly lakes such as lac Lusignan, attention to early warning signals such as the ones we measured here will be increasingly important to study as the possibility of eutrophication in more human impacted lakes accelerates.

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Microglia-Specific Genetic Factors in Autism Spectrum Disorder Etiology

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ABSTRACT Autism spectrum disorder (ASD) is a heritable condition that is associated with microglial activation and synaptic dysfunction. Microglia are the brain's resident immune cells in the brain which have been implicated in ASD pathology due to their role in synaptic pruning. Papers were selected based on inclusion criteria: 1) data on microglial genes, 2) ASD condition, 3) English language, and 4) genetic data available. The initial search strategy generated 372 articles from MEDLINE, Embase, Web of Science, and PsycInfo, after screening, 28 studies were included. Study design subsections were separated into 1) environmental, 2) experimental, and 3) case-control studies. CX3C motif chemokine receptor 1 (*CX3CR1*) and phosphatase and tensin homolog (*PTEN*) were implicated in the greatest number of studies with direct reference to microglial involvement. Studies screened predominantly supported the connection between microglial genetic variations and ASD symptomology: 13 experimental and environmental studies supported the ASD-microglial genetic connection and 4 were partially supportive; 8 human case-control studies were supportive and 2 were partially supportive. However, there was no consensus among studies regarding whether microglial up or down-regulation led to ASD symptomology. This review presents multiple novel microglial-genetic avenues such as the cyclical activation of the CX3CR1 through regionalized neural cytokine expression, the use of Gc macrophage activating factor (GcMAF) to normalize overactivated carbonyl reductase 2 (CBR2), the extensive effect of factors contributing to and resulting from maternal immune activation (MIA), and discrepancies between murine and human studies. These connections contribute to the web of ASD etiology and present targets for the development of ASD symptom management therapeutics.

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental spectrum disorder characterized by social deficits and repetitive behaviours that occurs more frequently in males (Faras et al., 2010; Hodges et al., 2020). People with ASD have often been noted to exhibit neuroinflammation (Morgan et al., 2010; Suzuki et al., 2013; Vargas et al., 2005) that may be responsible for some of the observed symptoms (Eissa et al., 2020). The immune system produces inflammation, which is caused by the release of pro-inflammatory factors like cytokines (Zhang & An, 2007). In the brain, the primary inflammatory immune cells are microglia, which are central nervous system (CNS) resident macrophage-like cells (Perry & Teeling, 2013). Microglia emerge from embryonic progenitors in early development and colonize the CNS. They mediate diverse neurological functions like CNS development and protection, neuroinflammation, and adult neurogenesis (Colonna & Butovsky, 2017; Nayak et al., 2014). Microglia play critical roles in healthy and pathological brain states and are a growing research area (Augusto-Oliveira et al., 2019; Ransohoff & El Khoury, 2015). Many studies have investigated the link between microglia and ASD concerning the effects of neuroinflammation on symptomology and pathophysiology (Rodriguez & Kern, 2011). This review explores the genetic factors that contribute to the microglial inflammatory state and its contribution to ASD symptomology.

In vitro research has demonstrated that ASD is linked to genetic markers, and alterations in the expression of microglia-specific genes can generate synaptic development and pruning issues (Andoh & Koyama, 2021; Cornell et al., 2022; Hodges et al., 2020). Synaptic pruning in the cerebral cortex removes unnecessary, old, and/or damaged synapses and reshapes synaptic connections (Faust et al., 2021). Children with ASD often have synaptic pruning deficits, leading to excess synapses and synaptic connections (Pagani et al., 2021). There are conflicting findings as to whether microglial overactivation increases or decreases ASD symptomology (Kim et al., 2017; Zhan et al., 2014). Two groups performed gene knockouts (KO) to the autophagy-related gene 7 (*ATG7*) gene and the CX3C motif chemokine receptor 1 (*CX3CR1*) gene, respectively (Kim et al., 2017; Zhan et al., 2014). Both genes facilitate synaptic pruning. *CX3CR1* promotes migration of microglia to the site of inflammation and pruning and ATG7 helps to induce neuronal autophagy (Kim et al., 2017; Zhan et al., 2014). *ATG7* and *CX3CR1* KO decreased microglia activation and decreased synaptic pruning capacity, resulting in ASD-like behaviours. However, it was previously discussed that an increase in microglial activation, leading to neuroinflammation, can also be a hallmark feature of ASD (Morgan et al., 2010; Suzuki et al., 2013; Vargas et al., 2005). Hence, further study in this area will shed light on the mechanistic differences in cases where microglia over- or under-activation can lead to ASD symptomology. (All abbreviations are summarized in Table 1).

METHODS

Papers were screened from 4 databases: Ovid MEDLINE (n=129), Embase (n=129), Web of Science (n=88), and PsycINFO (n=25). Table 1 contains the database-specific search terms. Inclusion criteria were: 1) data on microglial genes, 2) ASD-specified research area, 3) English language, and 4) available genetic (allotypic or genotypic) data. Studies were excluded if they were: 1) a review or systematic review, 2) a meta-analysis, 3) lacked controls, or 4) researchers did not provide access to the full-text article.

Following screening 372 studies were identified and 158 duplicates removed. During title and abstract screening, 95 studies were removed due to an absence of microglia-associated genetic data, did not study ASD, or were review articles. During full-text screening 91 additional papers were excluded due to lack of controls, focus on non-ASD conditions (such as Fragile X syndrome), or the authors could not obtain the full text from the researchers.

Table 1. Summary of abbreviations used throughout the review.

Acronym	Full Name
ASD	autism spectrum disorder
ATG7	autophagy related gene 7
CBR2	carbonyl reductase 2
CCL5	chemokine ligand 5
Chd8	chromodomain helicase DNA binding protein 8
CNS	central nervous system
CX3CR1	CX3C motif chemokine receptor 1
CXCL8	CXC motif chemokine ligand 8
CYP11A1	cytochrome P450 family 11 subfamily A member 1
DAP12	DNAX-activating protein of 12 kDa
DEP	diesel exhaust particulates
FPM	fine particulate matter
GABA-A	g-aminobutyric acid receptor
GcMAF	Gc Macrophage Activating Factor
GPR56	G protein-coupled receptor 56
HSP70i	inducible heat shock protein 70
IFN-γ	interferon gamma
IL-17a	interleukin 17a
IL-18	interleukin 18
IL-18r	interleukin 18 receptor
IL-1β	interleukin 1 beta
IL-37	interleukin 37
IL-38	interleukin 38
IL-38r	interleukin 38 receptor
IL-6	interleukin 6
KO	knockout
MeSH	Medical Subject Headings
mGluR5	Metabotropic glutamate receptor 5
MIA	maternal immune activation
MTOR	mechanistic target of rapamycin
NRF2	nuclear factor erythroid 2-related factor 2
Nrxn1	synaptic contact-dependent neurexin 1
NT	neurotensin
Oxtr	oxytocin receptor
P-Akt	phospho-Akt antibody
P2Y12R	platelet P2Y12 receptor
Psd95	postsynaptic density protein 95
PTEN	phosphatase and tensin homolog
Slc25a1	solute carrier family 25 member 1
TLR4	toll-like receptor 4
TMEM119	transmembrane protein 119
TNF-α	tumor necrosis factor alpha
TREM2	triggering receptor expressed on myeloid cells 2
Tsc2	tuberous sclerosis complex 2
TSPO	translocator protein
Zbtb16	zinc finger and BTB domain containing 16

RESULTS

Environmental Studies

Experimental studies utilized animal models and genetic modifications to investigate the role of individual genes in ASD symptomology. Studies utilizing gene KO models will be reviewed first. In this section mouse gene notation will be used. Three studies utilized global, in vivo, KO models to gain insights into the functions of oxytocin receptor (*Oxtr*) (Miyazaki et al., 2016), zinc finger and BTB domain containing 16 (*Zbtb16*) (Usui et al., 2021), and Metabotropic glutamate receptor 5 (*mGluR5*)(Chana et al., 2015) genes in ASD etiology. All of the models demonstrated increased ASD symptomatology; however, microglial involvement varied. *Oxtr* KO led to abnormal microglial activation and reduction of postsynaptic density protein 95 (*Psd95*) in vivo (Miyazaki et al.,

Table 2. Summary search terms used per database with notes and number of results gathered. Web of Science was refined by: Document type: articles AND excluding language: spanish

Database	Search Terms	Number of Results
MEDLINE	(microglia OR microgli*) AND ((asperger AND syndrome OR autistic AND disorder OR autism AND spectrum AND disorder) OR (autism OR asd OR autism AND spectrum AND disorder)) AND ((gene OR genetic OR genomic) OR (genetic AND transcription OR transcriptome)) AND (LIMIT-TO (PUBSTAGE , "final")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))	129
Embase	(microglia OR microgli*) AND ((asperger AND syndrome OR autistic AND disorder OR autism AND spectrum AND disorder) OR (autism OR asd OR autism AND spectrum AND disorder)) AND ((gene OR genetic OR genomic) OR (genetic AND transcription OR transcriptome)) AND (LIMIT-TO (PUBSTAGE , "final")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))	129
Web of Science	(microglia OR microgli*) AND (("Asperger Syndrome" OR "Autistic Disorder" OR "Autism Spectrum Disorder") OR (Autism OR ASD OR "Autism Spectrum Disorder")) AND ((gene OR genetic OR genomic) OR ("genetic transcription" OR transcriptome)) .ab,ti AND (LIMIT-TO (SRCTYPE,"j")) AND (LIMIT-TO (PUBSTAGE,"final")) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (LANGUAGE,"English") OR EXCLUDE (LANGUAGE,"French") OR EXCLUDE (LANGUAGE,"Polish"))	88
PsycInfo	AB=((microglia OR microgli*) AND ((Asperger Syndrome OR Autistic Disorder OR Autism Spectrum Disorder) OR (Autism OR ASD OR Autism Spectrum Disorder)) AND ((gene OR genetic OR genomic) OR (genetic transcription OR transcriptome))) OR TI=((microglia OR microgli*) AND ((Asperger Syndrome OR Autistic Disorder OR Autism Spectrum Disorder) OR (Autism OR ASD OR Autism Spectrum Disorder)) AND ((gene OR genetic OR genomic) OR (genetic transcription OR transcriptome)))	25

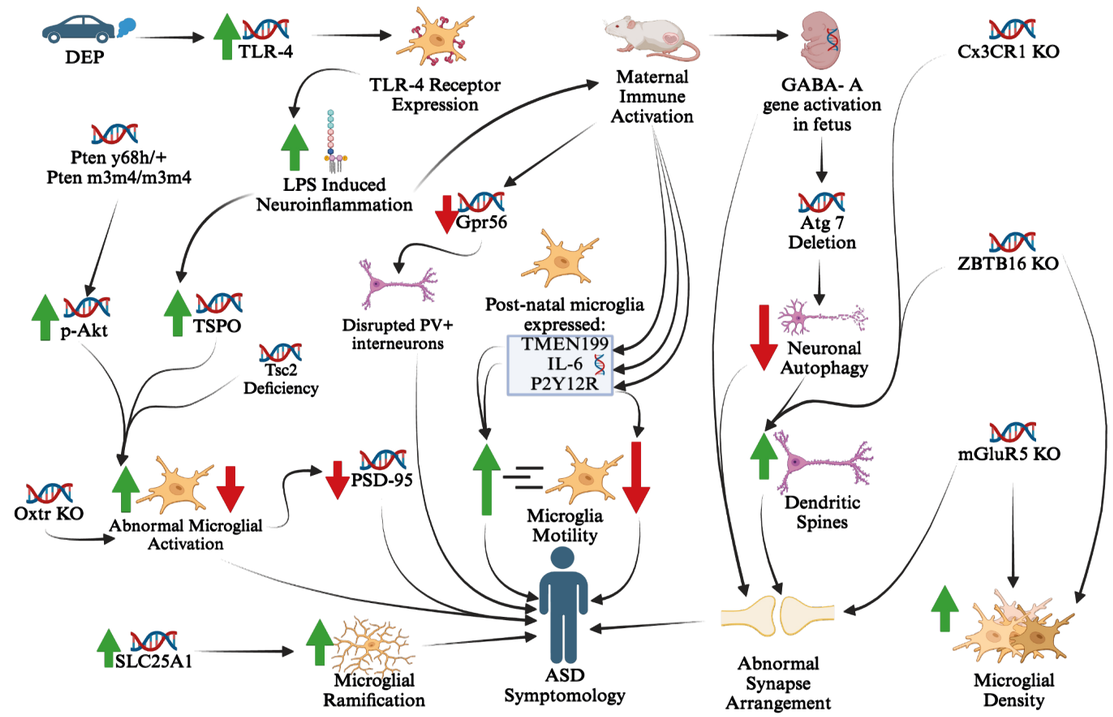

Figure 1. Schematic representation of directional gene expression in experimental models that support the microglial gene-ASD hypothesis and their correlations with neural and microglial cells. Many neuronal and microglial functions and behaviors have been associated with ASD symptomology. These include abnormal microglial activation, abnormal synapse arrangement, and modified microglial motility. This diagram demonstrates the correlational results of experimental studies linking gene expression to these ASD-associated neural and glial factors. The black arrows indicated the direction of theorized causality and potential pathways. The DNA symbol represents that the text near it is a gene. The bold green upward arrows indicate an upregulation of a gene, while the bold red downward arrows represent a downregulation. For any gene modification that is not up or downregulation, the modification is included in the gene text. DEP, Diesel exhaust particles; TLR-4, Toll-like receptor 4; PTEN, Phosphatase and TENsin homolog deleted on chromosome TEN; LPS, Lipopolysaccharide; p-Akt, phosphorylated protein kinase B; TSPO, translocator protein; TSC2, TSC complex subunit 2; OXTR, oxytocin receptor; PSD-95, postsynaptic density protein 95; SLC25A1, solute carrier family 25 member 1; TMEM199, transmembrane protein 199; IL-6, interleukin 6; P2Y12R, P2Y purinoceptor 12; GABA-A, γ-Aminobutyric acid sub-type A; ATG7, Autophagy related 7; CX3CR1, CX3C motif chemokine receptor 1; ZBTB16, Zinc Finger and BTB Domain Containing 16; GPR56, G protein-coupled receptor 56; mGLuR5, Metabotropic glutamate receptor 5; MIA, maternal immune

Table 3. Summary of controlled experimental studies and their examinations of microglia-associated gene expression in ASD disease state models. Each gene's function, independent of ASD, is listed along with its direction of regulation in the given ASD model. *:Partial support of ASD-microglia connection

Study ID	Model	Gene/Gene product	Gene Coding	Regulation Direction	Microglial association	ASD Impact
Chana 2015	mGluR5 KO mouse model	*mGluR5*	Codes for a glutamate receptor that is critical in synapse formation, and astrocytic postsynaptic excitatory regulation.	Downregulation	mGluR5 KO increased microglial density	Increased microglial density mirror ASD symptomatology
		Shank3	Codes for mGlu5 binding protein to positively regulate signaling	Downstream element of mGluR5		
		Plcb1	Activated by mGluR5 with roll in coordinating pre and post synaptic development	Downstream element of mGluR5		
Bolton 2017	TLR4-deficient pregnant females exposed to oropharyngeal aspiration of diseal exhaust particles	TLR 4	Sensing receptor for LPS and inflammatory response mediator	Upregulated by diesel exhaust particle (DEP) exposure	DEP exposure increases microglial activation and TLR4 expression. DEP exposed male microglia have closer neuron-interaction.	DEP alters microglial development and structure. Abnormal neuron-microglia spacing (in males) mirrors ASD brains.
Gassowska-Dobrowolska 2020*	Intraperitoneal injection of valproic acid to pregnant rats on genstational day 12.5	IL1β, IL6, tnf	Proinflammatory cytokines that mark the cytotoxic M1 microglial activation phenotype	Increased in valproic acid model	Markers of cytotoxic microglial activation	Valproic acid generated decreased communication and exploratory activity with no impact on social behaviour
		Arg1, Chi311, Mrc1, Cd86, Fcgr1a, Tgfb1, Sphk1	Anti inflammatory cytokine mRNA that mark the M2 cytoprotective microglia phenotype	Increased in valproic acid model	Markers of cytoprotective microglial activation	Valproic acid generated decreased communication and exploratory activity with no impact on social behaviour
		Shank2 &3	Integral for trans-synaptic connections, synapse stabilization, and synaptogenesis.	Increased in valproic acid model	Valproic acid induced increased IL-1b expression by microglia	Increased microglia-induce inflammation along with synaptic-related gene overexpression can cause disrupted synaptic structure and plasticity which could explain ASD-like symptomology
Kim 2017	Mouse model lacking atg7 gene in myeloid cells. Lyz2-Cre mice crossed with atg7fl/fl mice	*Atg7*	Important gene in normal neuronal autophagy	Down/ Deficient	Decreased spine density in Atg-7 deficient microglia. Increased synapse degradation and decreased brain connectivity	Associated with impaired social interaction and repetitive behaviour
Lee 2016*	Juvenile Wistar rat model of acute pain induced ASD. Subcutaneous injections of 5% formalin into both hindpaws of rat pups on post-natal day 3 through 5.	*Nrxn1*	Required gene for efficient neurotransmission and is involved in formation of synaptic contacts	Downregulation in males	increased inflammatory response, microglia count, and TNF-α and IL-1β	Partial association- cortical and hippocampal cell death observed
		Nlgn3	Important for synaptic formation and its mutation is associated with ASD	No change seen	increased inflammatory response, microglia count, and TNF-α and IL-1β	No direct ASD association
		Auts2	Implicated in neurodevelopmental conditions and developmental delay	No change seen	increased inflammatory response, microglia count, and TNF-α and IL-1β	No direct ASD association
Li 2018*	Male Shank3+/Δc mice aged 6-8 weeks of age. Tested for anethesia sensitivity using inhaled isoflourane.	*Shank3*	Codes for mGlu5 binding protein to positively regulate signalling	Downregulated in high dose particulate	microglia are not altered in Shank3 transgenic autism model, but are altered in particulate matter exposure	Correlated with increased ASD symptomology
		Cntnap2		No change seen	No effect	No effect
		Mecp2		No change seen	No effect	No effect
		Nrxn1		No change seen	No effect	No effect
		Fmr1		No change seen	No effect	No effect
Miyazaki 2016	Oxtr-deficient mouse model. Pregnant dams given munocycline mixed into drinking water while pregnant to interfere with microglial activation.	*Oxtr*	Associated with expression of postsynaptic density protein PSD95	KO- decreased PSD95 production	Abnormal microglial activation	Decreased mother-child communication as a symptom of ASD

Study ID	Model	Gene/Gene product	Gene Coding	Regulation Direction	Microglial association	ASD Impact
Ozaki 2020*	CX3CR1-EGFP transgenic pregnant mice were injected with intraperitoneal 10 mg/kg polyinosinic:polycytidylic acid. Observations taken in male mice day 18 of gestation and day 10 postpartum	CD68, ICAM-1, IL17a, Sall1	Cytokine and cell adhesion genes	No change seen	Shows there is not a morphological change associated with maternal immune activation	No effect
		Tmem119	Transmembrane protein only expressed by microglial cells not infiltrating macrophages.	Upregulated due to MIA	MIA not associated with microglial morphological changes but there is some postnatal microglial differentiation	Demonstrates some effect on MIA on microglial variation observed in ASD
		p2y12r	Only expressed by microglial not infiltrating macrophages. ADP receptor protein	Downregulated due to MIA	Gene specifically expressed in microglia	Demonstrates some effect on MIA on microglial variation observed in ASD
		Il-6	Cytokine gene- mediates MIA sequelae	Upregulated due to MIA	Increased motility of fetal microglia	Demonstrates some effect on MIA on microglial variation observed in ASD
Pan 2021	Preganant rat dams were injected with CYP11A1 gene-carrying adenoviruses on gestational day 8.5.	*CYp11a1*	Regulates GABAergic receptor expression	Upregulation	Microglial activation increased in overexpression model. Shown through increased TNF-α. Highly activated GABAergic synapse pathways.	CYP11A1 gene expression in pregnant rats could induce anxiety and autism-like behavior in their offspring through the regulation of microglial immune activation
Rigby 2022	Camk2a-tTA;TRE-SLC25A1 mouse model.	*Slc25a1*	Codes for mitochondrial solute carrier	Upregulation	Increased ramified morphology, causing disrupted white matter integrity and changed synaptic population	SLC25A1 overexpression led to autistic-like behaviours
Sarn 2021a	Mouse pten m3m4/m3m4 model	*Pten m3m4/m3m4*	Related to phagocytosis	Mutation/ Upregulation	Role in microglial activation, phagocytosis and synaptic pruning	No direct causative evidence found
Sarn 2021b	Mouse pten y68h/+ model	*Pten y68h/+*	Increases phosphorylation of Akt	Mutation	Increased microglial activation but not cell count.	Decreased preference for novel social stimuli, increased repetitive behavior, and increased thigmotaxis observed.
Shimoyama 2019	LPS-induced Tspo upregulation mouse microglial cell line BV-2	*Tspo*	Expressed by BV-2 cells when induced by LPS. Expressed in mitochondrial membrane	Upregulation upon LPS simulation	Expressed in microglia; recruitment of c-fos and c-jun have been found to increase the enhancer region of TSPO and causes microglial upregulation	Microglial activation, increased TSPO and neuroinflammation and related to ASD
Takanezawa 2021	Chd8 and Tsc2 deficent microglia from generated from C57BL/6J mice with lentivirus gene silencing	*Tcs2*	Negative MTOR regulator. Haploinsufficiency of this gene causes behavioural abnormalities related to ASD	Downregulation/ Knockdown	Reduced insulin-like growth factor after LPS stimulation. inhibited phagocytic activity. inhibition of microglia-mediated oligodendrocyte development	ASD related gene expression may be related to microglial role in oligo differentiation
		Chd8	Chromodomain- helicase DNA-binding protein. Haploinsufficiency of this gene causes behavioural abnormalities related to ASD	Downregulation/ Knockdown	Reduced insulin-like growth factor after LPS stimulation. inhibition of microglia-mediated oligodendrocyte development	ASD related gene expression may be related to microglial role in oligo differentiation
Usui 2021	Male Zbtb16 KO (B6.C3-Zbtb16lu/J) mice	*Zbtb16*	Regulates neurodevelopmental genes and myelination-associated genes	KO	Increased dendritic spines and microglia	Caused ASD-like social and cognitive impairment and repetitive behaviour.
Yu 2021	Gpr56f/f, Cx3cr1Cre/+, Gpr56 null (Gpr56f/f cross with CMVCre) and RosaGpr56 mouse models. MIA was induced in pregnant females using inraperitoneal injection of poly(I:C)	Gpr56	Related to late-stage progenitor proliferation	Downregulation in KO or Maternal immune activation model	Decreased microglial density and ramification and elevated TNF expression	Gpr56 down-regulation via MIA or Gpr56 KO impares late stage neurogenesis causing interneuron deficits and ASD-like behaviour
Zhan 2014	*Cx3cr1 KO mouse model*	*Cx3cr1*	Chemokine fractalkine receptor that is necessary for neuron-glial interaction	KO	Cx3cr1 KO= decreased microglia in postnatal period causing decreased synaptic pruning leading to ASD symptomology. Reduction of microglia reversed effects of gene mutation.	Seen to cause deficits in social interaction and increased repetitive behavior phenotypes associated with ASD

2016). In *Zbtb16* and *mGluR5* KO, the number of microglia and neuronal dendritic spines increased (Usui et al., 2021; Chana et al., 2015). This indicates that both the *Oxtr* and *Zbtb16* genes are integral to healthy synapse formation, and future research should explore links between the two (Table 3; Figure 1).

Genetic mutation or modulation was utilized in many of the experimental studies. For instance, it was observed that solute carrier family 25 member 1 (*Slc25a1*) gene upregulation in forebrain neurons is linked to increased microglial ramification in the hippocampus and ASD-like jumping behaviour in mice (Rigby et al., 2022). Repetitiveness and decreased sociability are other characteristics observed in murine ASD models, like the phosphatase and tensin homolog (*Pten*), *Pteny68h*/+ and *Pten* m3m4/m3m4 mouse models (Sarn, Thacker, et al., 2021, Sarn, Jaini, et al., 2021). Downstream effects of *Pten* expression include an increase in phospho-Akt antibody (P-Akt) and increased microglial activation and phagocytosis, which Sarn and colleagues hypothesized may be the cause of the ASD symptoms seen in the model (Figure 1) (Sarn et al., 2021). This is contradictory to the original theory that ASD stems from decreased synaptic pruning (Kim et al., 2017). However, approximately 10% of human cases of ASD can be traced to PTEN mutations, which indicates that at least some cases of human ASD can be attributed to increased synaptic pruning (Sarn et al., 2021). In conjunction with the original synaptic pruning hypothesis, reduction in microglia phagocytosis has been linked to ASD symptomology in tuberous sclerosis complex 2 (*Tsc2*) deficient models (Takenzawa et al., 2021). Tsc2 and chromodomain helicase DNA binding protein 8 (*Chd8*) have been associated with microglial involvement in ASD symptomology (Figure 1) and the deficiency of *Tsc2* or *Chd8* inhibits microglial-mediated oligodendrocyte development, which may lead to decreased neural myelination that is consistent with human ASD cases (Table 3) (Takenzawa et al., 2021).

Environmental factors such as exposure to diesel exhaust particulates (DEP) or maternal immune system activation (MIA) during pregnancy have been linked to the development of ASD. Lipopolysaccharide (LPS)-induced inflammation is frequently used as a model for environmental stressors in in vitro models. The toll-like receptor 4 (TLR4), present on the surface of microglia, acts as a bacterial LPS receptor and is produced by the *TLR4* gene that is upregulated by DEP (Bolton et al., 2017). LPS-induced inflammation has also been shown to upregulate the translocator protein (*TSPO*) gene. *TSPO* has roles in immune modulation and mitochondrial homeostasis, thus upregulation is hypothesized to activate microglia and induce ASD symptomology (Shimoyama et al., 2019). Interestingly, TSPO protein deficiency has been reported in human males with ASD, suggesting there may be additional complexity in translating the murine models to humans (Figure 1) (Table 3 & 4) (Zurcher et al., 2021).

In models of MIA, LPS is utilized to activate the γ-aminobutyric acid receptor (*GABA-A*) gene which mirrors the genetic expression observed in MIA (Estes & McAllister, 2016; Kirsten et al., 2015). This activation has been shown to generate variation in neuronal communication (Pan et al., 2021). Further transmission disturbances may be attributed to defects in parvalbumin-positive interneurons which are generated in MIA models via downregulation of microglial G protein-coupled receptor 56 (*GPR56*) gene expression in an interleukin 17a (IL-17a)-dependent manner (Yu et al., 2022). Models of MIA have noted upregulated transmembrane protein 119 (*TMEM119*) and interleukin 6 (IL-6) which have been shown to increase microglial motility (Ozaki et al., 2020) (Figure 1; Table 2). As demonstrated in these models external inflammatory factors can increase microglial activation and produce symptomology concurrent with ASD.

The final subset of experimental studies used an externally induced model of ASD to observe the effects on microglia genes. In the study conducted by Lee et al. the researchers utilized a formalin acute-pain-induced inflammatory response model of ASD to locate changes in the synaptic contact-dependent neurexin 1 (*Nrxn1*). They showed a male-specific decrease in *Nrxn1* and an increase in microglia count and inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) (Lee et al., 2016). However, no *Nrxn1* involvement was observed in a fine particulate matter (FPM) model of ASD. The FPM model had a related microglial alteration and SH3 and multiple akryin repeat domains 3 *(Shank3)* gene downregulation (Li et al., 2017). Inflammatory models, either via allergen- or asthma-induced MIA, were associated with microglial dysfunction and synaptic alterations. Differing models, including asthma, allergy, cytochrome P450 family 11 subfamily A member 1 (*cyp11a1*) overexpression, and cx3cr1 KO, caused synaptic variation (Saitoh et al., 2021; Ciernia et al., 2018). Microglia morphology was consistently implicated in synaptic dysfunction; however, the dysfunction was not unidirectional, and there were increased and decreased synaptic connections (Kim et al., 2017; Zhan et al., 2014). Although this does not provide a uniform explanation for ASD etiology, it highlights the importance of microglia and synaptic formation in ASD brain development (Table 3).

Children who grow up in urban areas tend to have a higher iwith microglial dysfunction and synaptic alterations. Differing models, including asthma, allergy, cytochrome P450 family 11 subfamilies A member 1 (*cyp11a1*) overexpression, and *cx3cr1* KO, caused synaptic variation (Saitoh et al., 2021; Ciernia et al., 2018). Microglia morphology was consistently implicated in synaptic dysfunction; however, the dysfunction was not unidirectional, and there were increased and decreased synaptic connections (Kim et al., 2017; Zhan et al., 2014). Although this does not provide a uniform explanation for ASD etiology, it highlights the importance of microglia and synaptic formation in ASD brain development (Table 3).

Case-Control Studies

Experimental models provide a unique look into the mechanisms of disease, however, these models do not always match what is observed in human cases. For instance, the deletion of *Cx3cr1* in microglia was shown to disrupt synaptic pruning in mouse models (Zhan et al., 2014). These results are contradictory to what is seen in human studies where *CX3CR1*, triggering receptor expressed on myeloid cells 2 (*TREM2*), and DNAX-activating protein of 12 kDa (*DAP12*) are upregulated in the ASD prefrontal cortices (Edmonson et al., 2014). In a study comparing ASD and healthy control blood or saliva, next-generation sequencing found a correlation between the Ala55Thr mutation of *CX3CR1* and decreased microglia-neuronal *CX3CR1* signaling in ASD (Ishizuka et al., 2017). However, the *Cx3cr1* KO mouse study showed that *Cx3cr1* KO decreased observable synaptic pruning and resulted in ASD symptoms (Zhan et al., 2014). Altogether, these studies demonstrate a disparity regarding *CX3CR1* association with ASD symptomology as *CX3CR1* KO (Zhan et al., 2014), upregulation

(Edmonson et al., 2014), and mutation (Ishizuka et al., 2017) all resulted in ASD symptomatology (Figure 2; Table 4). These results are likely a larger reflection on the differences between the mouse models of ASD and the human condition than on the role of *CX3CR1*/*cx3cr1* in ASD symptomology.

Many of the human studies found that people with ASD had dysregulation to genes that generated microglial inflammation in response to LPS. Considering that LPS is a bacterial protein this could link many of these genetic differences to MIA, which LPS can also trigger. For instance, a study in 2022 by Wang et al. demonstrated that children with ASD have a higher proportion of chemokine ligand 5 (CCL5), a monocyte chemoattractant, in their blood. This level was seen to increase in response to LPS-activated microglial mechanistic target of rapamycin (MTOR) signaling (Wang et al., 2022). Neurotensin (NT) stimulation can also increase MTOR signaling in microglia (Figure 2) (Patel et al., 2016). MTOR signaling activates microglia, however, these studies indicate that both infections, possibly MIA, or NT levels can trigger the pathway, leading to microglial secretion of inflammatory cytokines into regions of the brain. This neural inflammation may be a contributor to ASD symptomology. Two studies have demonstrated that in animal models interleukin 37 (IL-37) can reduce IL-1β and CXC motif chemokine ligand 8 (CXCL8) expression and NT secretion (Tsiloni et al., 2019; Tsiloni et al., 2020) (Table 4). Therefore, IL-37-like drugs, could synthetically aid in reducing negative ASD symptoms through the reduction of microglial inflammation.

Individuals with ASD express a higher level of inflammatory cytokine-regulated NT. Inflammatory cytokine expression is regionalized within brains of people with ASD. Cytokines IL-37, interleukin 18 (IL-18), TNF, and IL-18 receptor (IL-18r) are all upregulated in the amygdala and dorsolateral prefrontal cortex whereas interleukin 38 (IL-38) and IL-38 receptor (IL-38r) are decreased in the amygdala (Tsilioni et al., 2019)(Figure 2). Within the anterior cingulate cortex, proinflammatory major histocompatibility complex II cell surface receptor (*HLA-DR*) and cluster of differentiation 68 (*CD68*) are reduced in the grey matter. IL-1β is increased in the white and grey matter (Figure 2, Table 4) (Sciara et al., 2020). IL-1β, TNF-α, and interferon-gamma (IFN-γ) expression induce *CX3CR1* cascade activity (Table 3) (Zhang et al., 2010). This leads to a potential cycle within ASD etiology; *CX3CR1* increases microglial activation and proinflammatory cytokine release, which promotes further *CX3CR1* activation (Ishizuka et al., 2017).

Decreased levels of the nuclear factor erythroid 2-related factor 2 (*NRF2*) gene in microglia increase inflammation and cytokine release (Nadeem et al., 2020). Individuals with ASD have a lower level of *NRF2* transcriptional factor in their frontal cortices (Schrier et al., 2022). They have also been found to have upregulated levels of inducible heat shock protein 70 (*HSP70i*) and IL-6 which

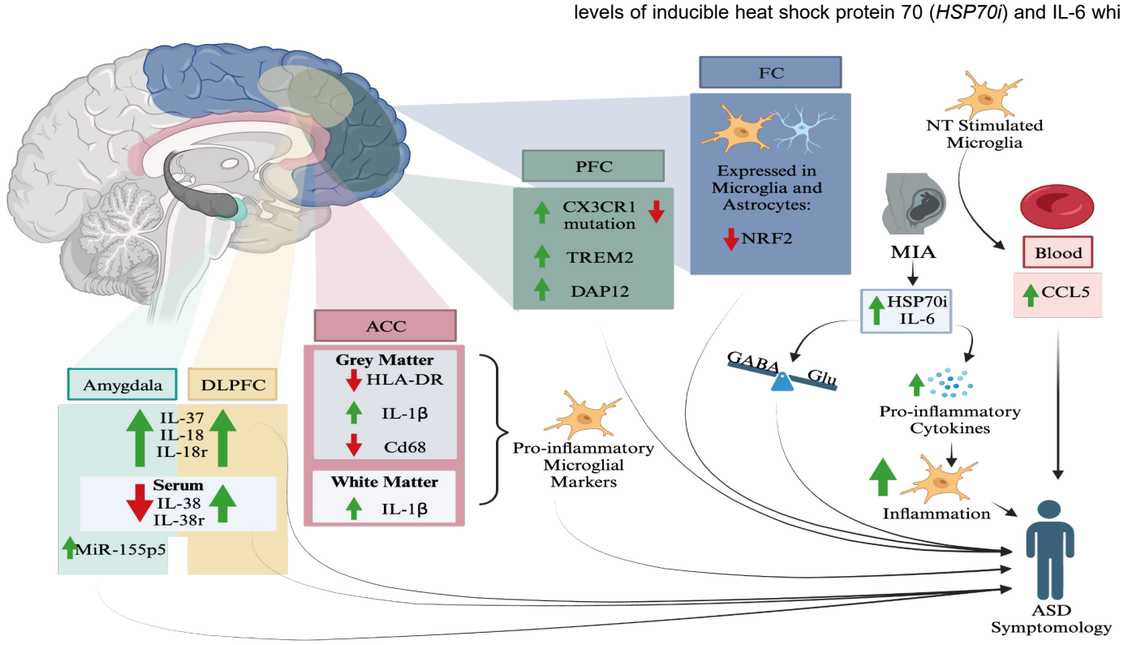

Figure 2. Schematic representation of directional gene expression by brain or tissue region observed in cases of ASD and their correlations with microglial cells. The figure summarizes the gene variations in different areas in the brain and blood observed in ASD cases. The black arrows indicated the direction of theorized causality and potential pathways to ASD symptomology. The colored boxes correspond with the color of the highlighted brain area or blood that they are associated with. Genes that are written spanning two boxes are expressed in both tissue areas. The bold green upward arrows indicate an upregulation of a gene, while the bold red downward arrows represent a downregulation. For any gene modification that is not up or downregulation, the modification is included in the gene text. The see-saw icon indicates an imbalance between the two neurotransmitters that is indicative of ASD. IL-37, interleukin 37; IL-18, interleukin 18; IL-18r, interleukin 18 receptor; IL-38, interleukin 38; IL-38r, interleukin 38 receptor; MiR-155p5, MicroRNA 155p5; HLA-DR, Human Leukocyte Antigen – DR isotype; IL-1β, Interleukin 1β; CD68, Cluster of differentiation 68; CX3CR1, CX3C motif chemokine receptor 1; TREM2, triggering receptor expressed on myeloid cells 2; DAP12, DNAX-activating protein of 12 kDa; NRF2, nuclear factor erythroid 2–related factor 2; MIA, maternal immune activation; HSP70i, human protein heat shock protein family A (Hsp70) member 1A; IL-6, interleukin 6; CCL5, chemokine (C-C motif) ligand 5; GABA, γ-Aminobutyric acid; Glu, glutamate; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; PFC, pre-frontal cortex; FC, frontal cortex; ASD, autism spectrum disorder; PV+, parvalbumin-positive.

Table 4. Summary of controlled experimental studies and their examinations of microglia-associated gene expression in ASD disease state models. Each gene's function, independent of ASD, is listed along with its direction of regulation in the given ASD model. *:Partial support of ASD-microglia connection

Study ID	Population	Gene	Gene Coding	Regulation Direction	Microglial association	ASD Impact
Abruzzo 2019	Children; ASD group (17 males and 4 females: mean age = 6.8 years) and TD group (14 males and 6 females: mean age 7.6 years).	*HSP70i*	Chaperon molecule involved in cellular defence mechanisms. Cytoprotective molecule expressed with PRX5 in a negative correlation with the expression of inflammatory cytokines	Upregulated	Increased with microglial inflammation	Increased inflammation and oxidative stress was found to increase clinical manifestations of ASD
		IL6	Plays role in CNS development, neurogenesis, and gliogenesis.	Upregulated	Increased gliogenesis, and increased Prx5 levels which are produced by microglia.	Increased inflammation and oxidative stress was found to increase clinical manifestations of ASD
Edmonson 2014	Human brain samples	*TREM2*	Receptor for Dap12 encoded microglial-specific transmembrane signalling polypeptide.	Upregulated	Microglia-specific expression	Increased expression in prefrontal cortex of ASD post-mortem brains.
		DAP12	Encodes microglial-specific transmembrane signalling polypeptide.	Upregulated	Microglia-specific expression	Increased expression in prefrontal cortex of ASD post-mortem brains.
		CX3CR1	Encodes for CX3C chemokine receptor 1	Upregulated	Microglia-specific expression	Increased expression in prefrontal cortex of ASD post-mortem brains.
Ishizuka 2017	192 ASD patients and 370 SCZ patients	*CX3CR1*	Encodes for CX3C chemokine receptor 1	Mutated-Ala55Thr	Receptor gene expressed solely in brain microglia	Ala55Thr mutation leads to disruption in CX3CR1 signalling increasing ASD symptomatology
Patel 2016	Control and NT-stimulated human microglia-SV40	*NTR3*	NT Stimulates Proinflammatory Cytokine and Chemokine Release from Human Microglia via NTR3/Sortilin	Increased NT leads to increased NTR3 expression in microglia	Increased microglial activation and increased gene expression of IL-1beta, CXCL8, CCL2, CCL5	Increased Levels of Serum NTR3/Sortilin Is Detected in the Serum of Children with ASD.
Schrier 2022	Human brains- 8 control 7 ASD	*NRF2*	Binds to antioxidant response element to control antioxidant gene expression	Expressed in microglia and astrocytes	Specifically expressed in microglia- increased SLC1A1 coupled with nfe2l2 gene expression	mRNA coding for the transcription factor Nrf2 is decreased in frontal cortex of ASD subjects. Expression of transculturation pathway genes controlled by Nrf2 is also decreased in ASD.
Sciara 2020*	Gray and white matter tissue in the anterior cingulate cortex from ASD and age matched typically developing (TD) control brain donors	*HLA-DR*	Pro-inflammatory gene	HLA-DR reduced in grey matter	Microglia expressed genes/cytokines	Association viewed in known ASD brains
		IL1β	Pro-inflammatory cytokine	IL1β increased in white and grey matter	Microglia expressed genes/cytokines	Association viewed in known ASD brains
		CD68	Pro-inflammatory gene	CD68 downregulated in grey matter	Microglia expressed genes/cytokines	Association viewed in known ASD brains
		NOS2	Pro-inflammatory gene	No difference seen for NOS2	microglia expressed genes/cytokines	Association viewed in known ASD brains
Siniscalco 2014*	22 ASD 20 control children- GcMAF treatment	*CB2R*	Codes for CB2R protein in blood monocyte-derived macrophages	Increased in ASD children- reduced by GcMAF treatment	CB2R is expressed in macrophages and microglia	Demonstrates role of endocannabinoid system in ASD

Study ID	Population	Gene	Gene Coding	Regulation Direction	Microglial association	ASD Impact
Tsilioni 2019	Postmortem human brain tissues of deceased Caucasian male children (3 to 14 y old) with ASD (n = 8) and non-ASD (n = 8)	IL-37	Anti-inflammatory cytokine	Increased in ASD amygdala and dorsolateral prefrontal cortex	IL-37 Inhibits NT-Stimulated Secretion and Gene Expression of IL-1β^2 and CXCL8 from Human Microglia	NT simulation of IL-37 microglia expression provides possible disease pathogenesis
		IL-18	Inflammatory cytokine	Increased in ASD amygdala and dorsolateral prefrontal cortex	Associated with neuroinflammation due to microglia	Demonstrates neuroinflammation in ASD
		TNF	Inflammatory molecule	Increased in ASD amygdala and dorsolateral prefrontal cortex	Associated with neuroinflammation due to microglia	Demonstrates neuroinflammation in ASD
		IL-1β	Proinflammatory marker that results from NT stimulation	Inhibited by IL-37	Expressed as proinflammatory cytokine by microglia	Demonstrates neuroinflammation in ASD
		CXCL8	Proinflammatory marker that results from NT stimulation	Inhibited by IL-37	Expressed as proinflammatory cytokine by microglia	Demonstrates neuroinflammation in ASD
		IL-18r	Proinflammatory cytokine receptor	Increased in amygdala and dorsolateral prefrontal cortex	Associated with neuroinflammation due to microglia	Demonstrates neuroinflammation in ASD
Tsilioni 2020	Brain samples (Caucasian male children age 3-14,8 ASD brains, 8 controls)	IL-38; IL-38R	Receptor for IL-38 that inhibits secretion of IL-1 from microglia when simulated by NT	Increased in ASD serum and decreased in amygdala	IL-38 inhibits activation of human microglia	Expression changes in ASD brains demonstrated known link
Wang2022	Blood Samples- 54 children with ASD and 20 control (74; 50 male and 24 female)	*CCL5*	Expression mediated via LPS induced mTOR signalling, gene expression dependent on NF- κB activation and CREB suppression.	Upregulation	Increase in CCL5 is caused by activation of mTOR signalling in microglia (suppressed by rapamycin mTOR inhibitor)	Observed in known ASD cases

have been linked to microglial inflammation and gliogenesis respectively (Abruzzo et al., 2019). Increased IL-6 levels may indicate prenatal MIA, and its dysregulation could be responsible for the neurotransmitter GABAergic-glutaminergic imbalance observed in ASD that has been attributed to MIA (Xu et al., 2020) (Figure 2; Table 4). Considering that *NRF2, HSP70i* and IL-6 dysregulation can generate microglial inflammation, these genes may contribute to the inflammatory cytokines seen in the prefrontal cortex (Tsilioni et al., 2019).

A 2020 paper investigated the expression levels of proinflammatory microRNA *MIR-155p5* in the brains of ASD and non-ASD children (Figure 2). This gene is important for immune cell proliferation and increases within microglia in response to LPS. The microRNA expression may provide a link to explore microglia and ASD pathogenesis, potentially with a link to LPS induced MIA (Table 4) (Almehmadi et al., 2020).

Abnormal carbonyl reductase 2 (*CBR2*) expression was seen in the macrophages and microglia of ASD children compared to typically developing children and was normalized by Gc macrophage activating factor (GcMAF). It is known (Siniscalco et al., 2014) that cMAF can oppose the impact of nagalase, the enzyme responsible for hindering the conversion of vitamin D3 binding protein to GcMAF. Nagalase is often increased in ASD and is associated with reduced macrophage activation, and GcMAF has been used with some success as a therapeutic in reducing ASD symptoms (Table 4) (Siniscalco et al., 2014).

DISCUSSION

People with ASD are often capable of living fulfilling and successful lives, with different perspectives and traits than their neurotypical counterparts. Therefore we do not propose the use of this review to treat ASD, but rather to understand the etiology and provide treatment for negative symptoms such as communication difficulties. The microglial genes *PTEN* and *CX3CR1* were presented most frequently in ASD studies. *PTEN* is a tumour suppressor gene that regulates the rate of cell division and has been shown to increase microglial activation and phagocytosis (Sarn, Jaini, et al., 2021; Sarn, Thacker, et al., 2021). ASD is associated with decreased synaptic pruning, and the characteristic symptoms of the condition have been generated in mice with gene KO. The *CX3CR1* gene was implicated in both mouse models and human cases. Although ASD symptomology was seen in both species the mouse models used a *cx3cr1* KO while human studies observed increased levels of *CX3CR1*. (Ishizuka et al., 2017; Zhan et al., 2014; (Edmonson et al., 2014). This may indicate a discrepancy between the animal studies used and their translation to human patients, it would be advantageous for researchers to see if this phenomenon exists within other murine ASD models.

The environment in which a child develops can influence the genes later expressed. Children who grow up in urban areas tend to have a higher incidence of ASD diagnosis compared to those raised in rural settings (Zeidan et al., 2022), which has been hypothesized to be due to a higher exposure to diesel exhaust particulates

(DEP). As demonstrated by Bolton and colleagues DEP leads to *TLR4* gene upregulation and increased microglial motility. It has also been suggested that there is a connection between the impaired movement of microglial cells and MIA. Specifically, the gene expression of ADP-receptor *P2Y12R*, which when increased has been found to decrease microglial movement. In contrast, the expression of specific transmembrane and cytokine genes *TMEM119* and IL-6 has been shown to increase their movement (Ozaki et al., 2020). *TMEM119* is a microglial marker following inflammation, and IL-6 is a proinflammatory cytokine, therefore it is logical that their increase would coincide with inflammatory microglial actions. The mechanism of MIA-induced genetic variation was hypothesized to follow epigenetic modifications (Ciernia et al., 2018). This was observed by modified gene methylation patterns in asthma-induced MIA (Ciernia et al., 2018). External factors such as pollutants or MIA was indicated to be an overarching influencer on genetic variations seen in ASD. Interestingly these external factors tend to be observed to have a larger effect in males (Bolton et al., 2017; (Lee et al., 2016); Lopez-Aranda et al., 2021). In the population, there is a larger proportion of males with ASD, which may be attributed to their sensitivity to external influences on their microglial genetic expression. When taken together with commonly noted genetic modifications, these environmental variations may provide an avenue for understanding the effects of external factors on the development of ASD and whether simple environmental adjustments could reduce the incidence of the condition. Another non-genetic factor that greatly influences the likelihood of developing ASD is dysbiosis of the gut microbiome. The gut biome of both the individual and the mother can contribute to ASD symptomology. This topic in ASD in general (Morton et al., 2023) and in the microglia–microbiome interaction (Davoli-Ferreira et al., 2021) has been well-reviewed elsewhere.

CONCLUSIONS

The review indicates that modulation of microglial genes can both induce ASD symptomology in mouse modes and are observed in human cases. However, murine and human studies did not always correspond, in particular to the directional expression of the *CX3CR1* gene. Environmental factors such as DEP and MIA can modify genetic expression in a male-predominate manner, and many of the genes investigated may have an MIA link via LPS activation. Continuation of research to develop a greater understanding of the interplay between microglia gene-modifying factors and their resulting pathophysiology will help in the understanding and treatment of ASD.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Effects of carbon dioxide fertilization and copper exposure on photosynthesis in hornwort

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ABSTRACT As global carbon dioxide (CO_2) emissions continue rising to unprecedented levels, photosynthetic efficiency of terrestrial plants is also increased. This phenomenon is known as CO_2 fertilization. While advantageous to aid in the removal of excess greenhouse gases, CO_2 fertilization may be offset by the simultaneous increase in heavy metal pollutants, such as copper, cadmium, lead, and mercury. The purpose of this experiment was to investigate whether heavy metal pollution, modelled through copper II sulfate ($CuSO_4$), may significantly impair CO_2 fertilization and photosynthesis in marine plants. We examined the volume of O_2 produced and the rates of photosynthesis in Ceratophyllum demersum (hornwort). Plants were placed in tubes of pre-boiled water with dissolved baking soda ($NaHCO_3$) as the CO_2 source. There were five treatment groups: no hornwort, hornwort control, hornwort + CO_2 fertilization, hornwort + $CuSO_4$, and hornwort + $CuSO_4$ + CO_2 fertilization. We found that CO_2 fertilization increased O_2 production and photosynthetic rate, while the addition of $CuSO_4$ inhibited photosynthesis and the positive effects of CO_2 fertilization. These results imply that although CO_2 fertilization can increase photosynthesis and eliminate some of the excess CO_2 in our atmosphere, this effect will be eliminated if we do not also control the amount of heavy metal pollution ejected into the environment.

INTRODUCTION

Climate change is a phenomenon caused by the frequent use of fossil fuels. When fossil fuels are burned, greenhouse gases, like carbon dioxide (CO_2) and methane (CH_4), are emitted into the atmosphere, trapping heat and reflecting solar energy back towards the Earth. Greenhouse gas emissions have surged to unprecedented heights; CO_2 alone has increased 100 times faster than what would be expected due to natural causes over the past 60 years (Lüthi et al. in 2008). In 2020, the global amount of CO_2 was reported to be 412.5 parts per million (Bhatt et al., 2023). This is far beyond what natural processes can remove, causing temperatures, ocean levels, and ocean acidity to rise. As temperature rises, ecosystems are destroyed, species are forced to extinction, and glaciers melt further, releasing more greenhouse gases and perpetuating the vicious cycle (Ruppel & Kessler, 2017). An area of interest to climate scientists is the natural reversal of human-induced production of CO_2 by photosynthesis. Photosynthesis uses CO_2 and solar energy to produce oxygen gas (O_2) and glucose (Equation 1).

$$6\ CO_2 + 6\ H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6\ O_2 \quad (1)$$

With the increase in CO_2 levels, scientists hypothesized that photosynthetic rates would also increase, since CO_2 is a crucial reactant in photosynthesis. Indeed, terrestrial photosynthesis has increased by 13.5% from 1981 to 2020, corresponding to a 17% increase in atmospheric CO_2 concentrations over the same amount of time (Keenan et al., 2023). This translates to an additional 14 peta-grams (1015 grams) of CO_2 removed from the atmosphere per year through terrestrial photosynthesis alone. This phenomenon is known as CO_2 fertilization (Kramer, 1981). Oceans are also well-established carbon sinks. They are responsible for recycling half of the global CO_2 (Field 1998, Behrenfeld 2001). Marine plant species contribute to approximately 30-40% of CO_2 assimilation each year (Mackinder et al., 2016). Accompanying the rises in CO_2 emissions, the extent of assimilation done by the ocean increased substantially from 1992 to 2018 (Watson et al., 2020). Thus, the increasing CO_2 levels in the ocean may also contribute to analogous CO_2 fertilization effects on the marine photosynthesis, providing greater opportunities for natural removal of CO_2.

Yet, there is another factor to consider; the industrial sources that release CO_2 into the atmosphere also release heavy metal pollutants that reduce chlorophyll a, b, and carotenoid in leaves by 17.84 - 48.73% (Joshi & Swami, 2009). A previous study investigating the relationship between CO_2 fertilization, photosynthetic rates, and the effect of pollutants found that while CO_2 enrichment accelerated the efficiency of CO_2 fixation pathways in chlorophyll, the presence of pollution, specifically heavy metals such as copper and zinc compounds, caused the greatest reduction of photosynthesis in six terrestrial plant species (Joshi & Swami, 2009).

To our knowledge, few, if any, studies have demonstrated a similar negative relationship between CO_2 fertilization and heavy metal pollution on marine plants. Thus, this study aimed to examine the effects of CO_2 fertilization and heavy metal (copper) pollution on the rate of photosynthesis in a marine plant, Ceratophyllum demersum (hornwort).

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METHODS

Plant species selection and sample preparation

We selected Ceratophyllum demersum (hornwort) as the aquatic plant because of its high growth rate and low requirements for light and CO_2. The hornwort (PetSmart, Richmond, British Columbia, Canada) was kept in an air-tight container sealed by parafilm (Bemis Company, Neenah, Wisconsin, USA) near a window with water refilled every day to keep it in good health. Samples were carefully taken from this specimen and blotted with a paper towel before they were weighed to make sure that treatment groups started with similar amounts of plant material. Dry masses of the samples were kept between 0.40 and 0.60 grams.

Treatments

Five different treatments were tested in 15 mL falcon tubes (Corning, Corning, New York, USA). For all treatments, 0.15 grams of baking soda dissolved in 5 mL of gasless water (boiled and cooled to room temperature) was used as the basal CO_2 source. Treatments were as follows: 1) No Hornwort Control. 2) Hornwort Only. 3) Hornwort + CO_2 fertilization; an additional of 0.10 grams of baking soda was added to simulate CO_2 fertilization after the first 30-minute interval. 4) Hornwort + $CuSO_4$; dissolved 0.10g $CuSO_4$ (ThermoFisher Scientific, Waltham, Massachusetts, USA) was added. 5) Hornwort + $CuSO_4$ + CO_2 fertilization; dissolved 0.10g $CuSO_4$ and an extra 0.10 grams of baking soda was added. All treatments were filled to 13.5 mL mark with gasless water. We chose 0.10 grams of baking soda for CO_2 fertilization so that the volume of CO_2 created from the baking soda would not entirely sequester the volume of O_2 produced from CO_2 fertilization. We also selected 0.10 grams of $CuSO_4$ to standardize the mass of additional treatments added, e.g., between baking soda and $CuSO_4$. Copper (from $CuSO_4$) was chosen as the heavy metal contaminant because its lower toxicity for handling and relevance in aquatic environments (Keller et al., 2017).

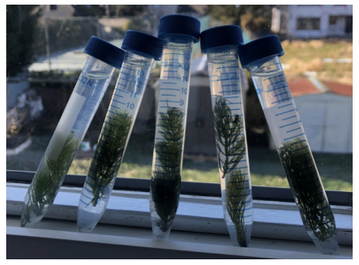

Figure 1. Representation of Treatments 2 and 3. A piece of hornwort was placed into a 15 mL Falcon tube. The addition of baking soda did not cause any cloudiness.

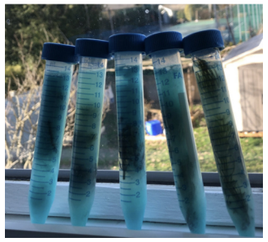

Figure 2. Representation of Treatments 4 and 5 ($CuSO_4$ treatment). A piece of hornwort was placed into a 15 mL Falcon tube. The addition of $CuSO_4$ resulted in cloudiness and a light blue tint to the water.

Prepared tubes were placed near a window to ensure sunlight exposure (Fig. 1, Fig. 2). Ten trials were performed for treatments two to five, with one tube for each treatment per day. The negative control was only recorded once. Experiments were conducted on days with moderate to high sunlight between 12:00 pm to 5:00 pm. Temperature ranged from 19.18°C to 20.22°C.

Oxygen gas measurement

Tubes were capped, sealed with parafilm, and inverted three times to ensure CO_2 equilibrium. Initial and subsequent volumes were recorded at half-hour intervals for a total of three hours. Volume changes were estimated from the height of liquid displacement with a ruler with 1 mm increments. The average initial rate (AIR), defined as the volume of O_2 produced for every 0.5-hour interval was calculated for the first 30 min of the experiment.

Data analysis

O_2 production and AIR data were recorded in excel (Microsoft, Redmond, Washington, USA) and graphed in R (version 4.3.3). Graphs represent the means ± standard deviation. A two-way ANOVA with type III sum of squares was conducted using GraphPad Prism (Boston, Massachusetts, USA). A Tukey post hoc multiple comparisons test was used to determine differences between treatment groups.

RESULTS

After 3 hours, hornwort with CO_2 fertilization produced the most O_2 (Table 1, 1.05 ± 0.04 mL). Hornwort alone produced about two-thirds as much O_2 (Table 1, 0.66 ± 0.03 mL). When hornwort was treated with $CuSO_4$, it produced significantly less O_2 than hornwort alone (Table 1, 0.23 ± 0.01 mL, $p < 0.0001$). CO_2 fertilization was not able to overcome $CuSO_4$ treatment and was not significantly different from $CuSO_4$ treatment alone (Fig. 3). The negative control (no hornwort) produced 0.03 mL of CO_2 gas (Fig. 3, Table 1).

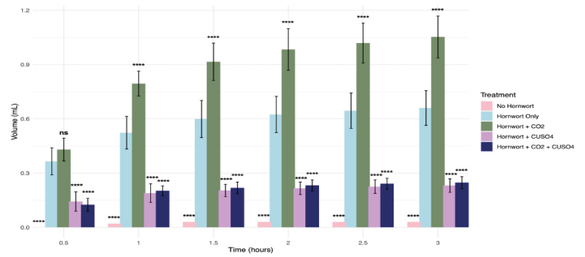

Figure 3. Average volume of gas produced over three hours (n = 1 for the no hornwort treatment, n = 10 for all other treatments). Error bars represent the standard deviation. Denoted significance refers to comparison to the hornwort only treatment (**** = $p < 0.0001$).

Table 1. Total O_2 synthesized for each time point and treatment group. Statistics refer to comparison to the hornwort alone treatment group for each time point. Statistically significant groups are **bolded**, insignificant groups are *italicized*.

	No Hornwort		Hornwort Only		Hornwort + CO2		Hornwort + CuSO4		Hornwort + CO2 + CuSO4	
Time (hours)	O2 production (mL)	adjusted p	O2 production (mL)	adjusted p	O2 production (mL)	adjusted p	O2 production (mL)	adjusted p	O2 production (mL)	adjusted p
0.5	0	**< 0.0001**	0.365	-	0.43	*0.2734*	0.143	**< 0.0001**	0.125	**< 0.0001**
1	0.02	**< 0.0001**	0.523	-	0.795	**< 0.0001**	0.189	**< 0.0001**	0.202	**< 0.0001**
1.5	0.03	**< 0.0001**	0.599	-	0.916	**< 0.0001**	0.204	**< 0.0001**	0.218	**< 0.0001**
2	0.03	**< 0.0001**	0.624	-	0.984	**< 0.0001**	0.216	**< 0.0001**	0.232	**< 0.0001**
2.5	0.03	**< 0.0001**	0.645	-	1.019	**< 0.0001**	0.225	**< 0.0001**	0.241	**< 0.0001**
3	0.03	**< 0.0001**	0.66	-	1.053	**< 0.0001**	0.231	**< 0.0001**	0.247	**< 0.0001**

Table 2. Rates of O_2 synthesis for each time point and treatment group. Statistics refer to comparison to the hornwort alone treatment group for each time point. Statistically significant groups are **bolded**, insignificant groups are *italicized*.

	No Hornwort		Hornwort Only		Hornwort + CO2		Hornwort + CuSO4		Hornwort + CO2 + CuSO4	
Time (hours)	AIR	adjusted p	AIR	adjusted p	AIR	adjusted p	AIR	adjusted p	AIR	adjusted p
0.5	0	**<0.0001**	0.365	-	0.43	**0.0002**	0.143	**<0.0001**	0.125	**<0.0001**
1	0.02	**0.0003**	0.523	-	0.795	**<0.0001**	0.189	**<0.0001**	0.202	**<0.0001**
1.5	0.03	**0.1871**	0.599	-	0.916	**0.022**	0.204	**0.0005**	0.218	**0.0007**
2	0.03	*0.9516*	0.624	-	0.984	**0.0325**	0.216	*0.9044*	0.232	*0.9459*
2.5	0.03	*0.9741*	0.645	-	1.019	*0.8783*	0.225	*0.927*	0.241	*0.927*
3	0.03	*0.9927*	0.66	-	1.053	*0.701*	0.231	*0.9736*	0.247	*0.9736*

The AIR of photosynthesis was 0.73 ± 0.05 mL/ hour for hornwort alone. Hornwort + CO_2 fertilization was 0.86 ± 0.04 mL/hour. In contrast, the AIR was 0.29 ± 0.03 mL/hour for hornwort with $CuSO_4$ and hornwort with $CuSO_4$ and CO_2 fertilization was 0.25 ± 0.02 mL/ hour. (Fig. 4, Table 2). Copper exposed treatments had significantly lower AIR and decreased to a near-zero rate at 1.5 hours, while the hornwort only AIR decreased to near-zero at 2 hours and the hornwort with CO_2 fertilization took around 2.5 hours.

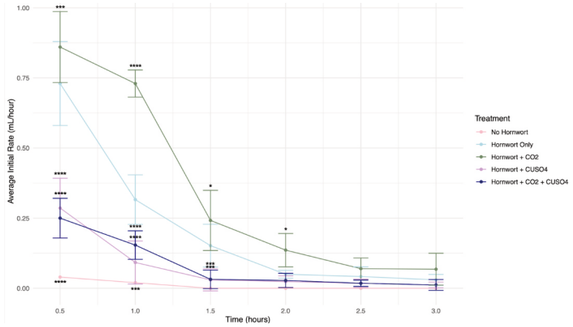

Figure 4. Rate of O_2 produced for each 0.5-hour interval (n = 1 for the no hornwort treatment, n = 10 for all other treatments). Error bars represent standard deviation Denoted significance refers to comparison to the hornwort only treatment (**** = $p < 0.0001$).

DISCUSSION

Here, we report that CO_2 fertilization in hornwort was effective at increasing photosynthetic capacity (volume of O_2 produced) and photosynthesis rates (AIR), and that the addition of $CuSO_4$ significantly impaired photosynthesis (Fig. 3, Fig. 4). According to these data, even if natural CO_2 fertilization occurs, it will not be beneficial for CO_2 removal if significant amounts of heavy metal pollutants, such as copper, are also present.

The volume of O_2 produced by photosynthesis with hornwort + CO_2 fertilization was significantly greater than that with hornwort alone (Table 1, Fig. 3). This result supports our hypothesis that CO_2 fertilization increases photosynthesis in marine plants, as it does with terrestrial plants (Keenan et al., 2023). The addition of $CuSO_4$ significantly reduced photosynthesis in hornwort (Table 1, Fig. 3). These results indicate that the inhibition of photosynthesis by $CuSO_4$ effectively attenuated the effects of CO_2 fertilization with lasting impacts. We chose copper (Cu^{2+}) as our heavy metal contaminant because of its relevance in aquatic environments; up to 95% of total Cu^{2+} released as pollutants is deposited in aquatic sediments (Keller et al., 2017). The rates and volume of O_2 synthesis decreased naturally in all treatment groups over time (Fig. 3, Fig 4). This is probably due to the saturation of photosynthetic activity or the exhaustion of CO_2 within the tubes (Wimalasekera, 2019).

Our results are similar to that of Jiang et al., 2010 who found that CO_2 fertilization enhances photosynthesis rates in Zostera capricorni (seagrass). According to Jiang et al., CO_2 fertilization enhanced the maximum electron transport rate. Our results also agree with those of Macinnis-Ng and Ralph, 2002 and Fernandes & Henriques, 1991, who reported that photosynthetic rates in seagrass decreased significantly in the presence of Cu^{2+}. Hypotheses for why the presence of Cu^{2+} may affect photosynthesis include compromising of enzyme activity, inhibiting pigment biosynthesis in chlorophyll, and leaf loss, which are all crucial for efficient photosynthesis (Muhammad et al., 2020). We did not see any leaf loss in our experiment.

There were three underlying assumptions that we made during our experimental design and data analysis. We first assumed that all the gases produced in the falcon tubes were O_2. This was a justified assumption because we first tested the control for the contribution of the CO_2 to the volume, as the $NaHCO_3$ will reach a solubility equilibrium. There was roughly 0.03 mL after dissolving, which was a negligible volume of CO_2 created.

The second assumption was that the conditions of light and temperature were constant, which allowed comparison of results across different treatments. We performed the experiment on a windowsill, instead of a tightly controlled lab setting, so temperature and amount of light was inconsistent over the period of data collection. Ceratophyllum demersum has a high tolerance for light intensity variability, so changes in sunlight in our trials likely did not substantially impact our results (Fair & Meeke, 1982). We also assumed that the blue colour and turbidity caused by addition of $CuSO_4$ did not impact rates of photosynthesis. Ceratophyllum demersum is markedly affected by environmental turbidity, however (Meyer & Heritage, 1941). So, it is possible that some of the reduction in photosynthesis that we observed could be due to the cloudy blue color induced by $CuSO_4$ (Fig. 2). A way to address this issue would be to use dyes to standardize the colour and turbidity between CuSO4 and water-only treatments. Another useful experiment would be to investigate dose-related effects of $CuSO_4$, which may also help clarify the influence of turbidity and copper concentration on photosynthesis.

The third assumption was that the variation in the masses of the plants [(0.40 to 0.60 g) ± 0.01g] did not significantly affect the amount of O_2 produced across treatments. The variation in plant leaf mass, when calculated over unit area, affects the photosynthetic efficiency of plants (Niinemets, 2001). However, the plant leaf mass per unit area measure was not measured in this experiment. In the future, we can quantify the plant leaf mass per unit area for each treatment to further control experimental parameters.

CONCLUSIONS

In summary, while efforts to combat climate change have focused on the reduction of CO_2 production, the reduction of heavy metal pollution may offer an alternative approach that is more effective and allows us to exploit the full potential of natural CO_2 fertilization. There is also evidence that copper nanoparticles could catalyze the reduction of CO_2 to convert it into an alternative fuel source (Xie et al., 2018). This opens new doors to transform malignant factors into something beneficial and convenient, allowing society to continue burning fuel while alleviating the stress on our environment. As Sir David King said, "The very last thing we should do is give up. I don't believe that is an option at all" (Seekings, 2021).

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