CANADIAN JOURNAL of UNDER GRADUATE RESEARCH

Melatonin

"Here we… argue that the effects and therapeutic potential of melatonin treatment against the negative impacts of cadmium toxicity on bone mineral density should be investigated. " (p.7)

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Sex, Gender, and Pharmacokinetics

"Current and future scientists should strive toward reducing health care disparities based on sex and gender in their studies, incorporating sex and gender-specific analysis whenever possible." (p.25)

Syrian Refugees' Mental Health

"With a sounder understanding of acculturation in Canada, we can better appreciate refugees' sense of urgency given the challenges of forced displacement from their homeland and inform the respective health, social policy, and practices to facilitate their successful transition." (p.14)

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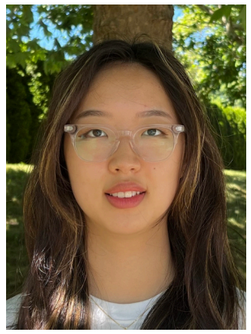
This issue is published on the traditional, ancestral, and unceded territory of the Coast Salish Nations, including xʷməθkʷəy̓əm (Musqueam), Sḵwx̱wú7mesh (Squamish), and səlilwətaɬ (Tsleil-Waututh).

Letter from the editors-in-chief

It is our utmost privilege to present to you Volume 8 Issue 2 of the Canadian Journal of Undergraduate Research (CJUR). This issue highlights another four exemplary articles written by hardworking undergraduate students at post-secondary institutions across Canada, containing important topics such as social factors in pharmacology, novel therapeutics, and the mental health of refugees. We at CJUR are incredibly proud to showcase such quality pieces of work put forward by undergraduate researchers.

This year, we have received 24 new manuscripts from undergraduate authors, and 300+ applications from potential graduate, postdoctoral, and faculty reviewers. We are incredibly grateful for the amount of support and contribution to our journal, as none of this would have been possible without the academic scholars putting their brilliant minds together.

As our academic year comes to a close, we would also like to acknowledge the tremendous efforts put forward by our editorial team. Our editors have worked tirelessly to ensure transparent and efficient communication with authors and reviewers, all while managing full-time jobs and/or study. It is because of our team that we were able to accept so many manuscripts and provide publishing opportunities to as many undergraduate researchers as possible. We would also like to thank our senior advisor and the presidents of UBC Undergraduate Research Opportunities (URO) for offering unconditional support.

We are very excited for you to read Volume 8 Issue 2, a culmination of years of work across Canada and a starting point for even greater research in the future.

Yours sincerely,

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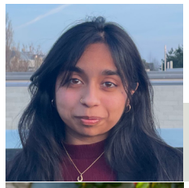

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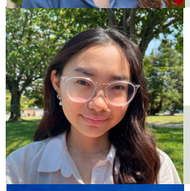

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Can melatonin ameliorate smoking-related cadmium-included decreases in bone mineral density?

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Abstract Cadmium, an environmental toxin, is associated with a range of adverse health effects including decreased bone mineral density and osteoporosis, due to its induction of oxidative stress, leading to DNA damage, mitochondrial dysfunction, and endoplasmic reticulum stress. Notably, cadmium is found at concentrations 4-5x higher in the blood of smokers versus non-smokers. Experiments performed in human cancer cells indicate that melatonin may directly protect against cadmium-induced tissue damage via regulation of mitochondrial activity. Further, recent evidence has demonstrated that melatonin can improve bone health for individuals with osteoporosis and partially protect against cadmium-associated inhibition of bone repair. Here we review these data and argue that the effects and therapeutic potential of melatonin treatment against the negative impacts of cadmium toxicity on bone mineral density should be investigated.

INTRODUCTION

A 2019 study estimated that there were 1.14 billion cigarette smokers globally, indicating that smoking remains a widespread risk factor to human health (GBD 2019 Tobacco Collaborators, 2021). Amongst the many negative health consequences associated with smoking, it is a known risk factor for osteoporosis and poor bone health, with one meta-analysis estimating the risk of osteoporotic fracture being 32% higher in smoking men and women compared to non-smokers (Kanis et al., 2005). Multiple mechanisms have been proposed that can potentially mediate this association, including the alteration of sex hormones and increased oxidative stress (Al-Bashaireh et al., 2018). Cadmium, an environmental toxin known to promote increased production of Reactive Oxygen Species (ROS) and mitochondrial dysfunction, is present in tobacco at concentrations ranging from 0.5-1.0 g of cadmium per cigarette, and is found at concentrations 4-5x higher in smokers versus non-smokers (Ganguly et al., 2018).

Longitudinal cohort and cross-sectional studies have established that exposure to cadmium is a risk factor for decreased bone mineral density (BMD) and osteoporosis. Experimental support for these epidemiological findings has shown that bone health, as measured by BMD, is highly sensitive to cadmium exposure even at levels as low as 0.3-10 mg/kg body weight (Buha et al., 2019), through both inhibition of the activity of osteoblasts, thus decreasing bone deposition, and stimulation of osteoclast differentiation, resulting in increased resorption and pit formation within bone (X. Chen et al., 2009, 2013; W. Liu et al., 2020; Ou et al., 2021). Ultimately, this results in the characteristic imbalance between bone deposition and resorption typically associated with the onset of osteoporosis in later life (X. Chen et al., 2009, 2013; W. Liu et al., 2020; Ou et al., 2021).

A study by Li and colleagues (2020) investigated how much the smoking-associated risk of osteoporosis was mediated by cadmium. Analysis of retrospective cohort data revealed that each 10-pack year (packs of cigarettes smoked per day, multiplied by the smoking duration, in years) could lead to 1.06 additional hip fractures per 1000 person-years, with 0.67 of this risk due to cadmium from tobacco smoke (Li et al., 2020). These results were supported by Elbeialy & Eldosouky (2018), who found an inverse relationship between serum and urinary cadmium and bone health. Thus, smoking is a known risk factor for poor bone health, with cadmium exposure being a relatively well characterized mediator of this risk. Despite this, no treatment or preventative measure has been identified that specifically mitigates the effects of cigarette smoking-associated cadmium-induced decreases in bone health.

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LITERATURE REVIEW

Intracellular cadmium toxicity

Much of the data regarding the mechanisms of cadmium toxicity has centered around its impacts on ROS production and mitochondrial function, where it is able to inhibit the coupling of the electron transport chain (ETC) and phosphorylation reactions, resulting in the inhibition of ATP synthesis within the mitochondria (Bradley et al., 1956), in addition to impeding succinate- and malate/pyruvate-stimulated respiration (Müller & Ohnesorge, 1984). Cadmium's direct role in Complex-I derived ROS production has been relatively well characterized. It has been demonstrated that cadmium binds to the Q-site of Complex I in the ETC or other NADPH dependent enzymes resulting in uncoupling (Cameron et al., 1986), and a significant increase in . ROS production (Hirst et al., 2008). Furthermore, the reduced Complex I activity results in electrons accumulating at the Q site and their transfer to molecular oxygen, forming $O_2\bullet-$ (Doughan et al., 2008). Cadmium has also been implicated in Complex III dysfunction; by inhibition of electron flow (Miccadei & Floridi, 1993) and binding to the Q-site of complex III preventing electron delivery from semi-ubiquinone to heme b_{566} causing semi-ubiquinone to accumulate and go on to donate the electron to O_2 forming $O_2\bullet-$ (Y. Wang et al., 2004). Similarly, Complex II is also thought to be a target for cadmium resulting in increased ROS formation, and playing a key role in cadmium induced cytotoxicity (Belyaeva, 2018).

In terms of ROS-independent mechanisms of cellular toxicity, cadmium also causes oxidative stress through intracellular depletion of glutathione (GSH), which acts as a key redox buffer within the cell, due to the fact that GSH contains thiol groups present in cysteine residues which cadmium has a strong affinity for (Nemmiche, 2017). Cadmium, like other heavy metals, can displace the zinc in thiols thereby disrupting the protein function (Bertin & Averbeck, 2006). Cadmium related depletion of GSH has been implicated in cell death of hepatocytes and primary oligodendrocytes (Almazan et al., 2000; Nemmiche, 2017).

Cadmium exposure has also been observed to up-regulate genes associated with cell cycle regulation, and proteins such as Growth Factor Receptor Bound Protein 2 (GRB2) and Shc adaptor protein, which are involved in cell proliferation and differentiation through the RAS pathway (Misra et al., 2003). Furthermore, it has also been shown that cadmium is able to compromise DNA integrity by inhibiting mismatch repair even at low concentrations such as 5 M through inhibition of ATP hydrolysis of mutS homolog 6 (MSH6) (Clark & Kunkel, 2004; Dally & Hartwig, 1997). Nucleotide excision repair is similarly inhibited by cadmium by reducing the DNA binding capacity of the xeroderma pigmentosum A protein thereby decreasing DNA damage recognition (Hartmann & Hartwig, 1998; Hartwig, 1998). Finally, base excision repair is impacted as cadmium exposure depletes human 8-oxoguanine-DNA glycosylase-1 protein (OGG1) by reducing the DNA binding activity of the transcription factor specificity protein-1 (Sp1) to the OGG1 promoter (Youn et al., 2005). Sp1 contains a zinc-finger motif whose cysteines may be targets for cysteine modification by cadmium, resulting in disruption of the protein. Additionally, high cadmium concentrations inhibit the nuclease activity of apurinic/apyrimidic (AP) endonuclease I which initiates repair of damaged bases (McNeill et al., 2004).

Cadmium toxicity in bone

As described above, cadmium exposure is associated with decreased BMD and osteoporosis, and many studies have examined the cellular and molecular mechanisms by which cadmium negatively affects bone health. Bone marrow mesenchymal stem cells (BMMSC) are multipotent stem cells capable of differentiation into osteoblasts (the cells responsible for producing new bone matrix), adipocytes, and chondrocytes (L. Hu et al., 2018). Rodriguez and Mandalunis (2016) demonstrated that cadmium decreased BMMSC viability, increased BMMSC differentiation into adipocytes, and relatedly decreased differentiation of BMMSCs into osteoblasts. Abnosi and Golami (2017) also reported that cadmium reduced BMMSC viability and proliferation and significantly reduced the concentration of intracellular calcium and alanine aminotransferase (ALT) and aspartate aminotransferase activities. Lv et al. (2019) demonstrated that cadmium interacts with BMMSCs through the receptor activator of the nuclear factor kappa B ligand (RANKL)/RANK/osteoprotegerin (OPG) signalling pathway resulting in suppression of osteogenic differentiation in vivo. This finding was supported by Knani and colleagues (2019) who also demonstrated that cadmium-induced bone damage mainly occurred as a result of suppression of osteogenic differentiation and relatedly caused an increase in adipocyte differentiation indicating the importance of osteoblast and adipocyte presence on bone health and homeostasis.

More recently, Hu and colleagues (2023) investigated the mechanisms behind cadmium induced inhibition of osteogenic differentiation in rat primary BMMSCs. Cadmium was observed to induce nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome pathways and resulted in autophagosome accumulation in BMMSCs, resulting in primary BMMSC cell death caused by impeded lysosome and autophagolysosome formation. Further, cadmium stimulated ROS/NLRP3/caspase-1/p20/IL- 1β inflammatory signalling pathways, causing BMMSC cell senescence and apoptosis. In addition, cadmium exposure resulted in the differentiation of primary osteoclasts and bone resorption activity. Finally, the authors also determined that Keap1/Nrf2/Are signalling is hindered which worsens oxidative stress within BMMSCs. They concluded that the toxicity of Cd in BMMSCs is induced through autophagy dysfunction and NLRP3 pathways (Hu et al., 2023). These results provide key insights into how cadmium impacts bone health at the biochemical level.

Differentiated osteoblasts are also a target for cadmium, which has been observed to induce osteoblast apoptosis. Zhao and colleagues (2015) determined that cadmium-induced apoptosis is mediated by the activation of caspase-3 and adenosine 5′monophosphate (AMP)-activated protein kinase (AMPK). Further, Liu and colleagues (2014) described that calcium-calmodulin-mediated mitochondrial dysfunction as well as cytochrome-C release hastened cadmium-induced apoptosis. As summarized by Ma and colleagues (2021), osteoblast apoptosis caused by mitochondrial dysfunction, endoplasmic reticulum stress, and oxidative stress, is a major component of bone health dysfunction because abnormal apoptosis can contribute to bone loss.

Cadmium is also known to affect the differentiation and activity of osteoclasts, the cells which are responsible for bone resorption. For example cadmium is observed to increase prostaglandin E2 (PGE2) synthesis and in turn stimulate increased bone resorption by osteoclasts (Collins & Chambers, 1991; Suzuki et al., 1989). A key mechanism that underlies greater osteoclast differentiation following cadmium exposure, as described by Chen and colleagues (2013) is that, following cadmium exposure, expression of RANKL increases while the expression of OPG, a decoy receptor for RANKL, decreases resulting in greater RANKL signalling and osteoclast differentiation.

Finally, a further study by Knani et al (2020) described how cadmium induced toxicity in rat bone was associated with reduced activity of glycogen synthase kinase 3- (GSK3), resulting in decreased levels of the proteins Wnt3a and -catenin. The Wnt/-catenin pathway is involved in the regulation of bone metabolism, where it induces the expression of osteoblast genes (Aida et al., 2018; Piters et al., 2008), mediating osteoblastogenesis as well as bone proliferation, differentiation, and mineralization (Baron et al., 2006; Glass et al., 2005; Mbalaviele et al., 2005). Rats exposed to cadmium showed a downregulation of osteogenic-related genes including Runx2, Ocn, and Alp (Knani et al., 2020). This downregulation of osteogenic genes caused by decreased Wnt3a and -catenin proteins, therefore results in the disruption of the careful balance between osteoblast bone deposition and osteoclast bone resorption, favouring a decrease in bone mineral density.

Preventing loss of bone mineral density

Widely used treatments for decreased BMD include anti-resorptive drugs and hormone replacement therapies (HRT). However, both these treatments have significant limitations. For example, anti-resorptive drugs can have significant side effects for the patient including osteonecrosis of the jaw and harsh repression of bone turnover (Brown, 2017; Kennel & Drake, 2009). Furthermore, HRT is used to balance estrogen levels within peri- and post-menopausal women to prevent the rapid bone loss common in post-menopause (Gambacciani & Levancini, 2014). HRT is not commonly used for women pre-menopause as estrogen levels in pre-menopausal women typically maintain serum estrogen above the threshold level required for maintenance of bone health (Stevenson, 2023). In addition, as reviewed by Gosset al (2021), there are associated risks with taking exogenous estrogen. Thus, HRT treatment is prescribed following an important risk-benefit analysis based on the type, dose and length of treatment as well as the woman's individual risk profile (Gosset et al., 2021). Adverse effects can include cardiovascular events, thromboembolism, stroke, and breast cancer (Rozenberg et al., 2020). Importantly, estrogens also mediate an important role in the bone health of men among other functions pertaining to fertility, fat formation, regulating insulin signaling, and regulating pancreas β cell function among others (Vandenput & Ohlsson, 2009). It has been described that men who are deficient in estrogen, for example those with aromatase deficiency due to a mutation in CYP19AI, or mutations in estrogen receptors that result in estrogen resistance, have delayed bone growth and unfused epiphyses (Morishima et al., 1995; Smith et al., 1994). However, men do not typically experience the same decrease in estrogen that women do with age meaning the addition of exogenous estrogen is unnecessary in most cases (Vandenput & Ohlsson, 2009). Furthermore, estrogen treatment in males is known to cause impaired development and function of the testes, prostate and seminal vesicles (Hammes & Levin, 2019; Masson & Selye, 1943). An additional important consideration is the cost-effectiveness of HRT. As reviewed by Rozenberg and colleagues (2020), menopausal hormone therapy (MRT) amongst females with a uterus was cost effective "only in those with a prior vertebral fracture" indicating that MRT use is not favorable in cases where fracture risk is low. This indicates that the use of hormone therapy as a preventative measure against bone loss caused by cadmium would be cost-ineffective, especially for those with financial restrictions. Thus, HRT is not a practical bone health prevention option for men or women pre-menopause. Cigarette smokers are present across the lifespan, found within both sexes, and are often chronic users/consumers. Thus, the existing treatments to improve BMD are insufficient to address the needs of this large and varied population of individuals.

Importantly, recent data has indicated that melatonin can protect against cadmium-induced oxidative stress (Hyun et al., 2023) and may attenuate the negative effects of cadmium exposure on bone repair (Luo et al., 2021). However, the efficacy of melatonin use in cigarette smokers and those regularly exposed to cadmium to preserve bone mineral density has not yet been characterized. Given this information, we propose that supplementation with melatonin could be an appropriate strategy for the prevention of bone density loss in individuals who are regularly exposed to cadmium by limiting oxidative stress and mitochondrial impairment.

Melatonin & bone health

Melatonin is a hormone synthesized and produced by the pineal gland (Cipolla-Neto & Amaral, 2018). The primary function of melatonin is in regulating the sleep-wake cycle and the modulation of circadian rhythms, and hence is a widely accessible pharmaceutical used to treat sleep-wake disturbances (Geoffroy et al., 2015; Tordjman et al., 2017; Xie et al., 2017).

Recent studies have found that melatonin also positively affects bone homeostasis and has been proposed as a potential treatment for osteoporosis/osteopenia (Amstrup et al., 2015; X. Wang et al., 2019). Melatonin is known to have strong antioxidant properties, which may in part explain its protective effects on bone (X. Liu et al., 2013; X. Lu et al., 2021; Tordjman et al., 2017). Melatonin's antioxidant capacities are exerted directly through its ability to scavenge free radicals and indirectly through activating antioxidative enzymes, inhibiting pro-oxidative enzymes, and tempering DNA repair pathways (Galano et al., 2018; Majidinia et al., 2017; Reiter et al., 2010). These mechanisms allow for melatonin to protect against free-radical-associated DNA damage (Galano et al., 2018). Furthermore, melatonin is thought to preserve the antioxidant capacity and bone-formation potential of bone-marrow-derived mesenchymal stem cells (BMMSCs) (W. Chen et al., 2020). In addition, melatonin has broader effects on bone, including promotion of osteoblast cell differentiation and type I collagen synthesis, thereby stimulating bone proliferation, and inhibition of bone resorption through the downregulation of RANKL-mediated osteoclast formation and activation (Koyama et al., 2002; X. Lu et al., 2021; Nakade et al., 1999; Xu et al., 2018). Within human populations, randomized control trials have found that melatonin supplementation is both well-tolerated and effective at improving physical symptoms in perimenopausal women with osteoporosis (Kotlarczyk et al., 2012); and was able to

increase bone mineral density at the femoral neck in a well-tolerated dose-dependent manner (Amstrup et al., 2015). Melatonin is also thought to be effectively non-toxic and is capable of improving circadian rhythm sleep disorders and poor sleep quality (Amstrup et al., 2015; Zisapel, 2018). Thus, melatonin is a safe, potential therapeutic for osteoporosis that is advantageous over other drugs, such as hormone replacement therapy and anti-resorptive drugs that often have significant side effects.

Melatonin & cadmium

Interestingly, previous observational and experimental studies have noted that cigarette smoking and third-hand smoke is associated with lower serum melatonin levels (Jiang et al., 2021; Ursing et al., 2005). Third-hand smoke is defined as the environmental hazard created via accumulation of second-hand smoke toxins on indoor objects (Jiang et al., 2021). Evidence indicates that polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke can increase the activity of cytochrome P450(CYP)1A2 (Ursing et al., 2005). CYP1A2 is associated with the breakdown of melatonin by the liver, which may explain the observed association between smoking and abnormally decreased serum melatonin (Ursing et al., 2005). However, other studies have found higher circulating daytime levels of melatonin in smokers (Tarquini et al., 1994). Thus, the association between smoking and melatonin levels remains to be clarified.

Recently, a study by Hyun and colleagues (2023) has revealed that melatonin directly counteracts the effect of cadmium on ROS levels within human prostate stromal cells and mouse embryonic fibroblasts. This is because melatonin enhanced the expression of mitochondrially-localized signal transducer and activator of transcription 3 (mitoSTAT3) that is reduced by cadmium exposure. MitoSTAT3 is thought to play an important role in the modulation of the electron transport chain (ETC), ROS homeostasis, transcription of mitochondrial DNA, ATP production, and apoptosis (Hyun et al., 2023). STAT3's role in ATP production has not been fully elucidated, however, mitoSTAT3 absence was shown to greatly decrease the activity of complex V in the ETC. Whether mitoSTAT3 affects the activity of Complex V or instead has some upstream effects on elements of the ETC is not yet fully understood (Gough et al., 2009; Meier & Larner, 2014). In addition, mitoSTAT3 maintains Complex I of the ETC's activity under ischemic conditions thereby restricting cytochrome c release (Szczepanek et al., 2011). This then preserves cell viability under cellular stress. Despite that STAT3's role in mitochondrial reperfusion injury is similarly not fully understood, STAT3 may modulate the mitochondrial permeability transition pore (MPTP) (Boengler et al., 2010). The MPTP is an important component in ischemia and reperfusion injury which remains opens under certain stimuli such as ROS and excess calcium, causing swelling of the mitochondria, mitochondrial dysfunction and culminating in apoptosis or necrosis (Halestrap et al., 2000). Tammineni et al (2013) determined using in-vitro studies that the gene associated with retinoid interferon induced cell mortality 19 (GRIM-19), a component of Complex I in the electron transport chain, acts as a chaperone to facilitate the recruitment of STAT3 into the mitochondria. GRIM-19 is also thought to increase the incorporation of STAT3 into Complex I (Tammineni et al., 2013). Notably, mitochondrial levels of GRIM-19 are altered by both melatonin and cadmium. Long-term exposure to CdCl2 decreased GRIM-19 levels in the mitochondria of WPMY-1 human prostate stromal cells (Hyun et al., 2023). Further, melatonin increases mitoSTAT3 levels following cadmium treatment, contributing to protection against ROS damage, mitochondrial dysfunction, and cell death (Hyun et al., 2023).

When specifically applied to osteogenic cells, Luo et al (2021) determined that pre-treatment with melatonin helped to maintain the integrity of the mitochondrial structure of BMMSCs and decrease DNA damage caused by cadmium within these cells, thereby protecting them against apoptosis. The authors conclude that melatonin may help to prevent cadmium-associated premature aging and apoptosis of BMMSCs (Luo et al., 2021). BMMSCs can differentiate into osteoblasts. Therefore, preventing cadmium-associated apoptosis of these cells, could increase osteoblast numbers (L. Hu et al., 2018). In addition, Knani et al (2019) determined that melatonin also protected against cadmium-induced accumulation of adipocytes within the bone marrow and concurrent metabolic disruption, most likely by maintaining the GSK2 kinase activity and promoting Wnt3a and -catenin signalling (Knani et al., 2020). As discussed above, cadmium encourages differentiation of BMMSCs into adipocytes while inhibiting differentiation into osteoblasts, resulting in dysregulated bone homeostasis. These findings suggest that increasing or maintaining the number of functional osteoblasts through preventative melatonin supplementation may help to rebalance bone deposition and resorption, thereby suppressing cadmium-associated loss of bone mineral density.

Thus, the use of melatonin in the prevention of cadmium-associated damage may be a mechanism to improve bone mineral density and osteoporosis outcomes within populations regularly exposed to cadmium, including smokers. However, the clinical relevance of melatonin supplementation in this population has yet to be explored. Given melatonin supplementation's well-tolerated nature and its potential to protect against cadmium-related exposure, examining this link may provide a new avenue for treating and preventing bone-mineral loss disorders such as osteoporosis within individuals who are exposed to cadmium.

Suggested Experimental Approach

We propose testing this intervention initially within human cell culture and animal models to better understand the effects of melatonin on cadmium induced decreases in bone mineral density in vitro. Previously, Luo et al (2021) used bone marrow-derived mesenchymal stem cells to determine that pre-treatment with melatonin was able to prevent cadmium-induced mitochondrial dysfunction and DNA damage, which are key impairments associated with cellular senescence. However, no studies have been done examining the effectiveness of melatonin treatment on osteocytes. To approach this, we suggest the use of monolayer osteocyte cell culture as described by Shah et al (2016) and osteocyte 3D organoid cultures, described by Knowles et al (2023) and Bernhardt et al (2020). Cultures could be pretreated with melatonin, then exposed to cadmium, in addition to separate cultures treated with melatonin following cadmium exposure to differentiate between these conditions. Analysis of key markers related to cadmium toxicity would include levels of ROS, mitochondrial function, endoplasmic reticulum function, autophagy, apoptosis (Ma et al., 2021) as well as ROS/NLRP3/caspase-1/p20/IL- 1β inflammatory signalling pathways and GRIM-19, Wnt3a and beta-catenin signalling as these have all been implicated in cadmium-induced stress on osteoclasts and/or BMMSCs (R. Hu et al., 2023; Hyun et al., 2023; Knani et al., 2020).

Following these cell-based experiments, animal models should be used to test the effectiveness of melatonin treatment in preventing cadmium-related bone damage in vivo. The protocol for exposure of mice to cadmium as described in Hu and colleagues (Hu et al., 2023) would be an appropriate model, with bone health quantified in accordance with Lu et al.'s (2021) procedure for using microCT and pQCT to determine bone mineral density. In addition, femoral and tibial extraction could be utilized to assess the bone mineral matrix, and oxidative stress markers including tiobarbituric acid reactive substances (Junqueira et al., 2021); as well as bone cytokines such as the RANK RANKL/RANK/OPG system for measuring osteoclast activity (Xu et al., 2018). Finally, osteogenic-related proteins Wnt3a/β-catenin and their associated genes including Runx2, Ocn, and Alp should also be analyzed (Knani et al., 2020).

If the cellular and animal model studies prove successful, this would pave the way for testing the efficacy of melatonin within randomized control trials that compare the effect of melatonin supplementation on bone mineral density, and fracture rate, across cigarette smokers of all ages. Additional consideration may be given to matched case-control trials that consider inclusion criteria such as age, number of cigarettes smoked per week, serum cadmium levels, or basal melatonin levels, given that endogenous melatonin levels can differ widely between individuals (Burgess & Fogg, 2008). Importantly, melatonin supplementation in smokers with unusually high melatonin at baseline may not provide any important benefit. As suggested by Tarquini and colleagues (1994), smokers may have supra-physiologically high levels of melatonin compared to their non-smoking counterparts. As a result, the signalling pathways that melatonin effects such as increasing the expression of mitoSTAT3 may become saturated (Hyun et al., 2023). As melatonin has been shown to help prevent age-related osteoporosis, these results could also be compared to the effectiveness of melatonin in preventing bone mineral density loss in age-matched non-smoking individuals.

Based on our proposed experiments we would expect to find that the loss of BMD over time within cigarette smokers supplemented with melatonin should be less pronounced than that of smokers who did not supplement for melatonin. We predict that this difference will result from increased expression of mitoSTAT3, which will in turn protect against the production of reactive oxygen species through modulating the electron transport chain (Gough et al., 2009; Meier & Larner, 2014) and the MPTP pore (Boengler et al., 2010), thereby facilitating the survival and function of osteocytes and osteogenic cells (Hyun et al., 2023). Furthermore, we would also expect the Wnt/-catenin pathway may play a key role in melatonin's reversal of cadmium toxicity as described by Knani at al (2020).

CONCLUSIONS

Cadmium, an environmental toxin known to cause decreased bone mineral density, is introduced into the body through cigarette smoking among other methods. To improve health outcomes for cigarette smokers, the authors propose the use of melatonin as a supplement. Melatonin is a widely used and well-tolerated over-the-counter pharmaceutical commonly utilized in the modulation of sleep-wake disorders. Importantly, new research has revealed that melatonin may be capable of attenuating ROS damage and mitochondrial dysfunction associated with cadmium exposure, as well as preventing apoptosis of BMMSC's. Importantly, melatonin supplementation would be a cost-effective and widely accessible treatment to prevent bone loss in smokers. Evidence supporting this strategy would present an option to improve health outcomes for millions of cigarette smokers globally and may overall reduce the burden of fractures related to loss of bone-mineral density. Thus, the proposal that supplementation with melatonin will prevent bone density loss in individuals who are regularly exposed to cadmium is one that should be considered for further clinical research.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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Syrian Refugees' Experiences in Canada and the Implications on Mental Health

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Abstract The Syrian refugee population represents an unprecedented number of migrants in Canada as vulnerable citizens and their families sought safety. Given the influx of refugees who were forced to leave their home country due to the ongoing civil war, the impact of adversities they experience as they transition to a new country cannot go unexamined. Thus, this review explores the implications on mental health that Syrian refugees experience throughout their integration process. Articles selected from a 2016 onwards depict the experiences of both Syrian children and adults by accounting for their traumatic experiences in Syria and their post-migratory experiences during their transition. Peer-reviewed qualitative, quantitative, and mixed-methods studies were considered in the con text of a thematic literature review. The results of the review includes two core themes: the significance of culturally appropriate healthcare services, highlighting the absence of inclusive healthcare that deter Syrian refugees from seeking necessary mental health treatment, and how Islamophobia serves as an obstacle in their integration. These discriminatory ideologies are a source of psychological distress for Syrian refugees and hinder their acculturation into Canada. This study discusses findings that shed light on such themes' implications for Syrian refugees and their mental health. Furthermore, this literature review addresses the significance of strategic social services, promoting the integration of Syrian refugees and positive mental health outcomes. The literature review, therefore, is particularly relevant since it positions us to positively respond to the current influx of Afghani refuges, among others. With a sounder understanding of acculturation in Canada, we can better appreciate refugees' sense of urgency given the challenges of forced displacement from their homeland and inform the respective health, social policy, and practices to facilitate their successful transition.

INTRODUCTION

Since 2011, political and military unrest in the Syrian Arab Republic has resulted in the displacement of millions of Syrian civilians (Guo et al., 2019). Canada was considered a safe destination for those impacted by the instability (Immigration, Refugees and Citizenship Canada [IRCC], 2022). Given the influx of civilians who were forced to leave their country due to civil unrest (Hadfield et al., 2017), the impact of the hardships experienced as they transition to a new country cannot go unexamined. Thus, this narrative review responds to an integral research question: what are the implications of barriers that Syrian refugees experience in their integration process? Undoubtedly, the exposure to violence and the loss of social supports during their pre-migration experiences hinders their ability to integrate in Canada (Yohani et al., 2019). The refugees' traumatic experiences often result in, or accentuates, mental health challenges and reduces their emotional functioning (Walker & Zuberi, 2020). It is especially noteworthy to explore how Syrian refugees' integration difficulties are linked to a greater likelihood of developing depression (Ahmad et al., 2021) and other mental illnesses (Almoshmosh et al., 2019). Understanding these post-migratory stresses and taking the steps to "improve the integration system" (Economou, 2019, p. 13) therefore serve as proactive responses to address mental health challenges (Almoshmosh et al., 2019).

The literature review examines the integration of Syrian refugees in Canada by accounting for their traumatic experiences in Syria and their post-migratory experiences during their transition in Canada in relation to the implications on mental health. In this context, successful integration is defined by Immigration, Refugees and Citizenship Canada as the process by which Syrian refugees become accultured (accustomed to Canadian culture) and active contributing members of Canadian society. For Syrian refugees in particular, the success of the integration process is related to governmental support and refugees' language skills (Government of Canada, 2019). The narrative review addresses the respective research question in a discussion of the two prevalent themes. First, the review considers the

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significance of culturally appropriate healthcare services for Syrian refugees' acculturation in Canada. Second, it discusses how Islamophobia and discrimination that many refugees experience hinders their integration.

It is imperative to consider the COVID-19 pandemic and its impact on mental health and mental health services. The global pandemic and government-mandated lockdowns imposed an additional set of challenges that disproportionately affected refugees in comparison to the general population. Syrian refugees were at higher risk for contracting COVID-19 given their lower socio-economic status and housing security (Clarke et al., 2021). Sharif-Esfahani et al. (2022) conclude that the pandemic contributed to a high prevalence of anxiety and fear of contracting COVID-19 among Syrian refugee populations residing in the Greater Toronto Area.

Rabiah-Mohammed et al. (2022) found that the challenges related to the pandemic reminded Syrian refugees of the hardships they encountered prior to their resettlement. Physical restrictions and social distancing measures prevented Syrian refugees from reuniting with extended family members, and hence, further accentuated their emotional distress. A participant in the study stated that, "we get sick of being alone. We do not see people; we are homesick; we do not see our family here. All of us have these feelings, we miss the family" (p. 18). Consider, too, that refugees self-reported feelings of financial constraint and marginalisation since they were unable to secure employment with sustainable wages, find adequate housing in safe neighborhoods, and develop language proficiency. This resulted in the perception that "the participants believed they were faring much worse than the general population in Canada" throughout the pandemic (Rabiah-Mohammed et al., 2022, p. 19).

The study's findings shed light on these themes' implications for Syrian refugees' mental health, justifying the need for this review. This is particularly timely not only because the Syrian refugee population represents an unprecedented number of migrants in Canada (Yohani et al., 2019) but also because of the Canadian government's commitment to welcome an additional 40,000 refugees from Afghanistan (Government of Canada, 2022). The findings of this review intend to offer more informed and inclusive services and proactively address the discriminatory practices experienced by newcomers (IRCC, 2021).

DISCUSSION/LITERATURE REVIEW

The findings of the narrative review include two key themes related to Syrian refugees' acculturation in Canada. This section discusses both themes in relation to mental health within the Syrian refugee population.

Culturally appropriate services and the implications on acculturation and mental health

Differences between Syrian and Canadian culture impede refugees' successful integration. Most refugees are unfamiliar with Canadian culture (Agroam, 2021) or the country's health and social services, collectively contributing to heightened stress throughout their transition (Agic et al., 2016; Ali-Hassan, 2021; Yohani et al., 2019). According to one refugee, "the most challenging things for me in Canada are the language and culture. Canadian culture is entirely different" (Aldibat et al., 2021, p. 494). Primitively, current literature identifies a lack of culturally sensitive health-related services provided for refugees in Canada, despite the importance of such services for successful integration (Agroam, 2021; Cheyne-Hazineh, 2020; Clarke et al., 2021; Sirin & Rogers-Sirin, 2015; Yohani et al., 2019). More specifically, the literature refers to the absence of culturally sensitive healthcare as a deterrent to Syrian refugees' likelihood to seek mental health treatment (Agroam, 2021; see also Walker & Zuberi, 2020). A culturally sensitive treatment approach in mental health services encourages healthcare providers to better understand the unique set of circumstances and needs of the Syrian refugee population. This treatment affirms the need to "sensitizing Canadians [in healthcare professions] to the ways in which culture can affect encounters between themselves and newcomers" in order to understand and respond to the "social detriments of health which builds on the resilience of refugee groups" (Agic et al., 2016, pp.6 & 7). Hansen & Houston (2016) discuss three levels of support services that are essential to provide a culturally sensitive treatment to the Syrian refugees. The first level (and similar to Agic et al., 2016) involves addressing the social determinants of mental health on a case-by-case basis. Secondly, healthcare providers are required to evaluate and recommend community supports programs for refugees. The third level includes psychosocial supports to assist refugees coping with uncertainties throughout the settlement process. In relation to Hansen & Houston (2016), Aldibat et al. (2021) discuss psychosocial services and culturally sensitive treatments that involve collaboration between health care providers, settlement and other community agencies to address the challenges experienced by Syrian refugees.

In addition, relevant literature suggests that the stigma associated with mental illness among the Syrian refugee population contributes to their reluctance to access mental health treatment (Agroam, 2021; Cheyne-Hazineh, 2020; Tuck et al., 2019). Hansen & Huston (2016) argue that the stigma surrounding mental health amongst Syrian refugees not only prevent them from seeking treatment in culturally unfamiliar spaces but contribute hesitancy when discussing their emotional well-being. In fact, Cameron et al. (2022) suggests that many avoid "culturally insensitive" medical practices as they accentuate distrust, existing stigma, and as a result, prevent future interactions with the healthcare system. According to Mahajan et al. (2021), many Syrian women in Canada are reluctant to seek mental health treatment as it is perceived as invasive and distressing. In sum, the perception of cultural differences in healthcare services contribute to health disparities between Syrian refugees and the Canadian population. Specifically, higher rates of unmet health-related needs among Syrian refugees compared to the general population (Tuck et al., 2019) and their greater vulnerability to poorer (physical and mental) health outcomes (Clarke et al., 2021).

This review will explore the stigma surrounding mental health perceived by Syrian migrants in Canada that contribute to their reluctance when seeking treatment for mental health (Almoshmosh, 2019; Cheyne-Hazineh, 2020) and contribution to a deep sense of shame and embarrassment during mental distress (Agroam, 2021). According to Abo-Hilal & Hoogstad (2013), this stigma may be the result of Syria's al-Assad regime that opposed the establishment of mental health services, contributing to its inaccessibility for Syrian citizens. President Bashar al-Assad's reign of power accentuated the Syrian population's lack of trust of

formal organizations, including healthcare services, due to "decades of oppression, widespread corruption and involvement of secret police in every aspect of life in Syria" (Abo-Hilal & Hoogstad, 2013, p. 92). In this way, the reluctance to trust established government organizations and services may be an aggravating factor that further undermines Syrian refugees' perceptions of culturally unfamiliar services offered in Canada and hence negatively impact their assimilation into Canada (Walker & Zuberi, 2020).

Also, processes related to mental health screening have significant implications for refuges. Given the stigma associated with mental illness and culturally unfamiliar services, comprehensive screening and mental health assessments immediately following their arrival can compromise emotional well-being, accentuate distress, and problematize their acculturation (Almoshmosh, 2019; Eggerston, 2016; Hansen & Huston, 2016). More precisely, literature states that "it is important to not over-diagnose Syrian refugees with clinical disorders, since their experiences cannot be described using Western-based, evidence driven medicine" (Cheyne-Hazineh, 2020, p. 132). Note that Almoshmosh (2019) specifically cautions that "diagnostic labelling" (p. 23) of mental illnesses for Syrian refugees should be avoided because such diagnoses are heavily stigmatized and "a source of shame and embarrassment" (p. 24). Additionally, Almoshmosh (2019) states that receiving psychiatric diagnoses may be especially shameful for men, not only because of stigma surrounding mental illnesses but also because of the culturally- embedded expectation that men cannot demonstrate weakness.

The discourses in the literature regarding diagnosing mental illness within the Syrian refugee population in Canada is conflicting. Eggertson (2016), who recognizes that psychiatric evaluations potentially can retraumatize patients with post-traumatic stress disorder (PTSD), proposes that symptoms of mental illness should be evaluated 3 to 12 months post-migration rather than immediately following their arrival. In this way, their privacy is further respected and they are provided the opportunity become acclimate to the new environments before evaluated for psychiatric illnesses (Eggertson, 2016). Conversely, Agroam (2021) argues that it is essential for Syrian refugees in Canada to be assessed for mental health symptoms "as soon as possible to avoid the severity of the condition" (p. 6), suggesting that early recognition and treatment is beneficial for well-being, quality of life, and eases their transition into Canada. These conflicting perspectives in the literature related to the diagnostic assessments of mental illnesses for the Syrian migrants underscore its complexity.

Implications of Islamophobia and discrimination on acculturation and mental health

Islamophobic and discriminatory practices have adverse implications for Syrian refugees' integration in Canada. Islamophobia is defined as the fear and hatred, and hostility directed towards Muslims, which in conjunction, give rise to prejudice and discriminatory practices (Çakı & Gülada, 2018). Syrian refugees and the broader Muslim community have been recognized as targeted groups for Islamophobia (Bose, 2022; Keung, 2016), commonly perceived "as an inherently violent religion and political ideology; seeing all Muslims as religious radicals or fanatics; and seeing all of Islam and Muslims as inherently mistreating women" (Wilkins-Laflamme, 2018, p. 90). The general Canadian population views migrants as being "out of place" because the latter ostensibly lack the common values of Western culture (Economou, 2019; Hynie, 2018; Wilkins-Laflamme, 2018). Relevant literature argues that Islamophobic perceptions of Syrian refugees contributes to their othering, which is associated with Saidian notions of Orientalism (Elkassem et al., 2018; Hynie et al., 2018) that promote a perception of non-Western cultures as inferior to the dominant West (Kyriakides et al., 2018), thus threatening the so-called ruling class's security and safety (Arif, 2018). Such ideologies not only create psychological distress for many Syrian refugees but significantly challenge their acculturation process (Economou, 2019; Furquan et al., 2022; Khan & Hamilton, 2019; Walker & Zuberi, 2020; Wilkins-Laflamme, 2018).

Additionally, the polls cited in the literature underscore the notion that Islamophobic attitudes are prevalent in Canada. For instance, more than half of the respondents to a 2015 survey believe that the Canadian government provides too much support for "underserving" Syrian refugees entering the country (Walker & Zuberi, 2020). Donnelly's (2017) survey of the Canadian population concluded that only 40% of respondents accepted the resettlement of Syrian refugees in Canada, while 36% and 24% were either indifferent or supported its ban, respectively. The prevalence of Islamophobia in Canada is also supported by a 2019 Ipsos survey that revealed that nearly half of the respondents admit to having racist thoughts against Muslims, and a quarter believe it is becoming increasingly acceptable to display prejudicial behaviour against Muslims (Mirrlees, 2021). This finding is concerning given that the number of police-reported hate crimes against Muslims has "more than doubled" between 2013 and 2016 (Mirrlees, 2021, p. 856; see also Furquan et al., 2022). According to one victim, "wearing the hijab can be hard. People might bully you [and ask] 'what is that piece of garbage on your head" (Elkassem et al., 2018, p. 12). Moreover, Islamophobic discourse may create an "atmosphere of contempt, mistrust and ignorance" (Helly, 2012, p. 3) that not only increases Syrian refugees' "marginalization and discrimination but in turn creates the structural conditions for poor health outcomes" (Economou, 2016, p. 3). Consequently, Syrian refugees are segregated from Western culture, thereby serving as a preventive factor in their acculturation (Wilkins-Laflamme, 2018).

The literature also points to instances of discrimination in the Canadian educational system, as many Syrian refugee students are targets of Islamophobic practices (Furquan et al., 2022; Guo et al., 2019; Tyyskä et al., 2017). The Islamophobic perceptions that exist across schools and schooling systems are especially pertinent considering that "schools often mirror the issues and discourses prevalent in the broader local and national community" (Walker & Zuberi, 2020, p. 404). Refugee students are subject to both physical and verbal violence in schools (Tysskä et al, 2017), and are disproportionately targeted against (Chuang, 2010; Drolet & Moorthi, 2018; Elkassem et al., 2018). The literature identifies that Islamophobic behaviour and microaggressions against refugee students contribute to an unwelcoming school environment and fuel lower self-esteem and feelings of disempowerment and alienation (Elkassem et al., 2018; Fazel et al., 2012; Guo et al., 2019). As a result, Syrian refugees who encounter discrimination are more likely to develop anxiety disorders, PTSD, and exhibit aggressive behaviour (Beiser & Hou, 2016). Fazel et al. (2012) suggest that experiences of discrimination for migrants may "continue to affect the mental health of refugees even 9 years after arrival" (p. 270). In turn, exposure to Islamophobic ideologies and practices in the educational context also mitigates their academic success (Chuang, 2010; Elkassem et al., 2018; Tysskä et al., 2017; Walker & Zuberi, 2020). Sirin & Rogers-Sirin (2015) support the evidence that high prevalence of discrimination in educational contexts adversely influences Syrian refugees' educational achievement and graduation rates. The negative impact on the students' engagement and learning represents a significant impediment to their education, integration, and resultingly, their mental health (Tysskä et al., 2017).

While Islamophobic views influence the perceptions of Canadians and permeate educational contexts, literature also identifies mainstream media as a weapon that shapes the discriminatory perceptions of their audiences (Walker & Zuberi, 2020). As major sources of information, the media can contribute to close-minded views and promote hostility towards refugees in Canada (Economou, 2019; Wilkins-Laflamme, 2018). According to Hynie (2018), it is common for media outlets to portray Syrian refugees as barbaric and uncivilized. Elkassem et al. (2018) noted that approximately 40% of the media that addresses Muslims and related Muslim-based issues convey negative connotations, and nearly two-thirds of such platforms associate this demographic with extremism. In addition, there are accounts of the media trivializing the concerns and needs of the Syrian refugee population (Hynie, 2018; see also Walker & Zuberi, 2020). The media that frames Syrian refugees as being a threat to public safety and security (Economou, 2019) depicts them as terrorists that must be ostracized from the Canadian population (Elkassem et al., 2018).

The literature also cites the gendered portrayal of Syrian refugees in Canadian media. Tysskä et al (2017) undertook a thematic qualitative media analysis of several hundred news articles (including sources from the CBC, Toronto Star, CTV, and National Post) to examine Syrian refugees' migration in Canada. The study revealed that across Canadian media platforms, Syrian men are commonly portrayed as terrorists that threaten the Western identity (see also Wilkins-Laflamme, 2018). Tysskä et al's (2017) finding supports Mirrlees's (2021) suggestion that Syrian men are represented as "terrorists-in-waiting or terrorists-in-becoming" (p. 868). In addition, Tysskä et al. (2017) noted that Syrian women and children were portrayed as vulnerable, with limited self-agency and that this discourse is consistent throughout the literature. Helly (2012) suggests that Syrian and Muslim women "are often represented as victims and alienated agents" and are mistreated by men (p. 6). Syrian women are underrepresented in Canadian media and are described as "extras in their own story," while being portrayed as vulnerable and desperate for humanitarian aid (Tyyskä et al., 2018, p. 158). Hynie (2018) states that the media facilitates the perception that Syrian refugees are not wholly Canadian citizens and are "a threat to [the] security, social order, and economic stability" of Canada (p. 2). Such portrayals contribute to antagonization and ostracization of refugees in Canada, which has direct and consequential repercussions on their successful acculturation and mental health (Economou, 2019; Hynie 2018; Mirrlees, 2021; Tuncer & Ebanda de B'béri, 2020).

LIMITATIONS

The narrative review investigated topics that have been examined by various researchers across diverse and varied disciplines; thus, a complete narrative review of every article related to Syrian refugees' experiences in Canada is beyond the scope of this study (Wong et al., 2013). Moreover, the sources under review were predominantly from peer-reviewed academic journals and written in English. It is possible that they may have been subject to selection bias.

CONCLUSIONS

The refugee resettlement experience is characterized by uncertainty, stress, and separation (Fazel et al., 2012; Oudshoorn et al., 2020; Yohani et al., 2019). Migration is not only considered a pervasive long-term experience (De Haene et al., 2007) but also one that exposes refugees to dangerous circumstances (Fazel et al., 2012). In fact, for those fleeing violence in their homeland, it is described as a stress-inducing and retraumatizing experience (Ahmad et al., 2021). This narrative review sheds light on the refugees' experiences of acculturation and integration in Canada, and the implications on their mental health. The research identified that trauma experienced by Syrian refugees during their pre-migration can undermine their ability to assimilate to Canadian culture successfully. Two key themes are identified in the literature review: culturally appropriate services and the effects and implications of Islamophobia and discrimination on refugees' acculturation.

This review addressed the importance of providing targeted social services to promote the integration of Syrian refugees and positive mental health outcomes. Discourse in relevant literature highlight that the differences between Syrian and Canadian culture have implications for Syrian refugees' acculturation and mental health in Canada. Furthermore, it is evident that instances of discrimination experienced by Syrian refugees hinder their acculturation and further accentuate any pre-existing mental health concerns. The narrative review provides necessary insight into the challenges that Syrian refugees encounter during their transition into Canada, and just as significantly, the implications for their mental health.

Consequently, the literature review is especially timely for various reasons. By acquiring a greater appreciation and understanding of acculturation in Canada, we are better positioned to positively respond to the current influx of Afghani refugees. Fleeing the oppressive regime of the Taliban, Afghani refugees share similar circumstances and challenges as their Syrian counterparts. Both populations have been forcibly displaced from their homelands and seek refuge in Canada with a sense of profound urgency. The analysis of the research under examination, then, can be used to inform current policy and practices regarding health and social-related supports and the prevention of discriminatory practices often experienced by these marginalized groups.

Several recommendations stemming from the review may be especially relevant. Policymakers, provincial and territorial ministries of health need to account for Syrian refugees' cultural nuances in the context of health-related services. Policy should identify the key principles of culturally inclusive healthcare and integrate specific objectives to meet the unique needs of Syrian refugees. For medical practitioners, the policy can bridge tangible culturally responsive health care practices that aim to address the

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stigma associated with mental illness for Syrian refugees. Through both policy and practice in federal, provincial and municipal governments, along with police services and the education of ministry, the Canadian system can work in tandem to raise the awareness of the devastating social, psychological, and physical impact of Islamophobia. These services and agencies serve to reach a broad audience and underscore the adverse societal consequences of discriminatory practices.

The themes discussed in the review not only lend themselves to the above recommendations, but also shed light on avenues for future research. Research on other countries and jurisdictions that welcome refugees and implement targeted and successful culturally inclusive healthcare services can inform the specific policy and practices across Canada. Relatedly, research into anti-discriminatory interventions can identify practices that have far-reaching positive societal outcomes. Just as significantly, ongoing research into the mental health outcomes of Syrian refugees in Canada provide more accurate depiction of refugees' long-term health. These longitudinal studies may evaluate the differential experiences between Syrian refugees of varying age and gender in order to investigate the similarities and differences in their experiences.

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CONFLICT OF INTERESTS

The author declares no conflicts of interest.

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Electrophysiology of Benign Familial Neonatal Seizures and the Current Therapeutic Approach

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Abstract Every year, 5 million people worldwide are identified for having epilepsies, with neonatal seizures accounting for approximately 2 per 1000 cases of term infants (Epilepsy, n.d.; Krawiec & Muzio, 2023). Specifically, some patients with a history of benign familial neonatal seizures were found to be more likely to get epilepsies in their later life. However, due to the high ethical standards being imposed on research involving neonatal populations, neonatal seizures and their anti-convulsant treatments are not as well-understood as other seizures. This is problematic, as the neonatal seizures should not be treated based on adjusting doses of conventional anticonvulsants for adults. This approach is not favorable as neonates have distinctive physiological characteristics that can be different from adults. Thus, simply adjusting the dose of the drugs may have sub- or supra-therapeutic effects, or even lead to lethal effects on the neonatal patients. The focus of this paper is to explain the electrophysiological cause of benign familial neonatal seizures and the therapeutical attempts that had been done to treat the syndrome.

INTRODUCTION

Benign familial neonatal seizure (BFNS) is a rare epilepsy syndrome. This disease typically occurs the first few days from birth, yet the patients generally do not display irregular electrical activities on development, cognition, and interictal electroencephalogram (EEG) records (Bayat et al., 2021). However, in some cases, focal or multi-focal readings were noticed on interictal EEG, as BFNS induces clonic seizures (Singh & Raj, 2008). Singh & Raj (2) also noted that ictal EEG can initially present short periods of electrical activity cessation just before the emergence of abnormal spikes and wave readings. These abnormal readings usually last for a few minutes (Singh & Raj, 2008).

BFNS is an inheritable disease. It is passed down to family members in an autosomal dominant trend with about 85% penetrance (Maljevic & Lerche, 2014; Plouin & Kaminska, 2013). The term "benign" in BFNS was given to its name, due to good clinical outcomes generally witnessed in these patients (Plouin & Kaminska, 2013). In many cases, the disease was found to be reversible; the patients could naturally remit to retain normal cognitive functions without the need for any pharmacological interventions (Maljevic & Lerche, 2014). For instance, in one family study including 69 BFNS patients, 68% of them remitted epileptic episodes within 6 weeks from birth (Tharp, 2002).

The rarity of this disease could partially be associated with this benign aspect of the disease. Singh & Raj (2008) pointed out that the family members of BFNS patients may expect spontaneous remission of the disease in their neonatal patients, based on their family history of BFNS. The family members may be less likely to request medical support for their neonatal patients. Or, often the episodes of seizures in the disease itself may be deemed as supernatural events in low- and middle-income countries (Singh & Raj, 2008). By treating BFNS as a minor disease or supernatural event, there could have been fewer reports of the incidences.

There is a need to scientifically investigate BFNS, as 15% of the recovered patients develop later episodes of epilepsy after remission (Panayiotopoulos, 2005). For instance, there is a limited understanding of the genetic or environmental causes of BFNS (Maljevic & Lerche, 2014). Also, in the same family study with the 69 patients, described above (Tharp, 2002), 16% of the BFNS patients had another onset of seizures around the age of 8 years. Additionally, out of these patients, 50% kept having more seizures in older age. Another study also found that 40% of its 10 BFNS families with private mutations on potassium ion channel receptors had slower psychomotor and cognitive maturity than other families without private mutations (Steinlein et al., 2007).

Currently, there is not much understanding on the BFNS and its treatment. For the scope of this paper, the ion channel defects related to the cause of BFNS and current pharmacological approach to the disease will be discussed.

DISCUSSION

Defective ion channels in BFNS

Human brain activities rely on ion channel functions, as it functions based on the electrical activities in its regions. Each type of channel establishes membrane potential and thereby determines the electrical activity. Hence, changes in the ion channels of brains can disrupt normal electrical activity, causing nearby neurons to simultaneously change their activities which can lead to epilepsies (Anwar et al., 2020).

BFNS is caused by a single gene mutation on the KCNQ2 gene in chromosome 20q13.3 or the KCNQ3 gene in chromosome 8q24 (Fister et al., 2013). The mutation can express defective potassium channels that lead to the disease (Fister et al., 2013). The KCNQ2 and KCNQ3 genes encode for the tetrameric voltage-gated potassium channels, Kv7.2 and Kv7.3 channels, respectively (Maljevic & Lerche, 2014; Tharp, 2002). The Kv7.2 and Kv7.3 channels are located in central nervous system, specifically in axon initial segments (part of axon for stimulating action potentials) and at nodes of Ranvier (part of axon transmitting action potentials) (Maljevic & Lerche, 2014; Wulff et al., 2009). Expression of either genes can translate into homomeric receptors, while the co-expression can result in heteromeric receptors (N. A. Singh et al., 2003). The heteromeric receptors can exhibit different potency from homomeric receptors to different anticonvulsant drugs (Maljevic & Lerche, 2014).

In the receptors, each tetrameric subunit is composed of six transmembrane proteins, and both amino and carboxyl terminus (C-terminus) are located intracellularly (Figure 1). The C-terminus of each subunit consists of regions to bind regulatory proteins and are involved in attaching to other subunits (Maljevic & Lerche, 2014). The fourth segment of each subunit (S4) has positively charged arginine residues, and it serves to detect voltages (Maljevic & Lerche, 2014). The sequence between the fifth and sixth segments (S5-S6) changes the shape upon the identified voltage difference on S4 (Maljevic & Lerche, 2014). This conformation change in S5-S6 can alter the pore formation intracellularly (Maljevic & Lerche, 2014).

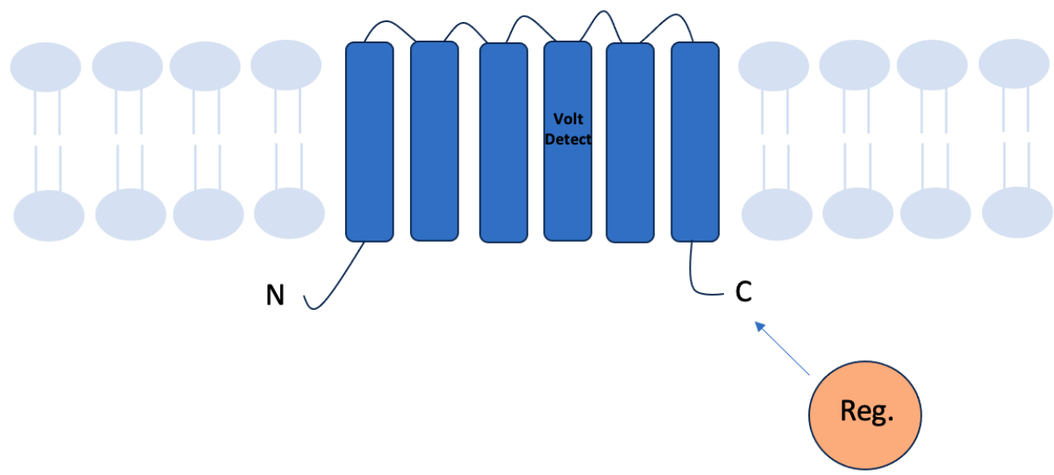

Figure 1: Voltage-gated potassium channel subunit. Regulatory protein (annotated as "Reg.") binds to C-terminus of each subunit, and fourth segment of the transmembranes (annotated as "voltage detect") has a positively-charged arginine residue for its function as a voltage detector.

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Normally, the Kv7.2 and Kv7.3 receptors open at voltages below the action potential threshold and are responsible for establishing muscarinic current (M-current), a "noninactivating slow [potassium] current" (Maljevic & Lerche, 2014). This M-current is important in preventing rapid firing of action potentials, and many pharmaceutical industries tried to discover muscarinic agonists to modulate the current and ultimately the synaptic plasticity (Maljevic & Lerche, 2014; Tharp, 2002; Wulff et al., 2009). Both homomeric and heteromeric receptors can generate M-current, but it was found that the M-current produced by the heteromeric receptors was at 10 times the magnitude than by the equally co-expressed homomeric receptors in an in vitro model (Maljevic & Lerche, 2014).

Over 60 mutations in the KCNQ2 gene and 6 mutations in the KCNQ3 gene were identified in families with BFNS to cause the disease (Heron et al., 2007; Maljevic & Lerche, 2014). In one study, Heron et al (10) discovered that 44% of the participants from their 9 family cases had deletions or duplications in the KCNQ2 gene. Moreover, roughly 50% of the mutations in the KCNQ2 gene lead to a loss of a large number of the last amino acids in the sequence, generating shorter subunits for the potassium receptors (Heron et al., 2007; Tharp, 2002).

The single gene mutations in the KCNQ2 and KCNQ3 genes lead to a loss of functions in both the homomeric and heteromeric channels (Kv7.2/Kv7.3) (Maljevic & Lerche, 2014; N. A. Singh et al., 2003). The mutations can elicit the loss of functions involving various mechanisms. For instance, a single gene mutation in the genes can lead to changes in gating functions, lower receptor expression, or generation of mutant proteins altering the normal receptor functions (Maljevic et al., 2008). The severity of the loss of functions may differ, as the effects can be complete or partial (Heron et al., 2007). Generally, the complete loss of function leads to the production of drastically smaller M-current, while the partial loss of function leads 20-25% decrease (Maljevic et al., 2008). Yet, this 20-25% reduction is a change in magnitude in the M-current great enough to trigger epilepsies (Maljevic et al., 2008).

In BFNS, the mutations could be found in Kv7.2 receptors at the C-terminus, S5-S6 sequence, S4 voltage-sensing segment, and S1-S2 region (Figure 2a). On the other hand, the mutations were in Kv7.3 receptors at the S5-S6 sequence (Figure 2b) (Maljevic & Lerche, 2014). The mutations in the C-terminus can disrupt the assembly of the subunits to form the channel (Maljevic & Lerche, 2014). Also, the mutation at the C-terminus can impede the transportation of the receptors to the brain membrane (Maljevic & Lerche, 2014). In the S4 segment, the mutations in the positively charged arginine residues lower the likelihood of detecting the change in voltage (Maljevic & Lerche, 2014). However, the mutations in other residues of the S4 segment alter the gating and thus conductance of potassium ions (Maljevic & Lerche, 2014). Congruently, the mutation (E119G) in the S1 to S2 region can increase the chances of action potential firings, most likely due to its close ionic interaction with S4 arginine residue (Maljevic et al., 2008; Wuttke et al., 2008). The mutations in S5-S6 segments, the domain essential to create an opening for potassium ion conductance, were found to be more prone to cause patients resulting with harmful clinical phenotypes, such as intellectual disability (Steinlein et al., 2007).

Although other ion channels (e.g. voltage-gated sodium or calcium channels) are known to be located near potassium channels, it is only the mutations in the voltage-gated potassium channel genes known to be directly associated with the cause of BFNS so far (Berkovic et al., 2004; Kannan et al., 2023). However, interestingly, the voltage-gated sodium channel gene SCN2A mutation on chromosome 2q24 was found to be associated with benign familial neonatal-and-infantile seizures, which typically occur later in infants around 11 weeks of age (Berkovic et al., 2004; Striano et al., 2006).

Pharmacological treatment for BFNS

Despite the self-limiting nature of the disease, neonatal seizures are important to be diagnosed and treated, as they may end up with unfavorable health outcomes (Spoto et al., 2021). The most effective antiepileptic drugs to treat neonatal seizures including BFNS are not well understood. Although neonatal seizure happens to approximately 3 out of every 1000 newborns (Panayiotopoulos, 2005), there are not many drugs approved to conduct the necessary clinical research on neonates (Spoto et al., 2021). This is most likely due to ethical and safety concerns in performing scientific research on newborns. Therefore, not much information on the complications and efficacies of neonatal antiepileptic drugs is available for clinicians to practice.

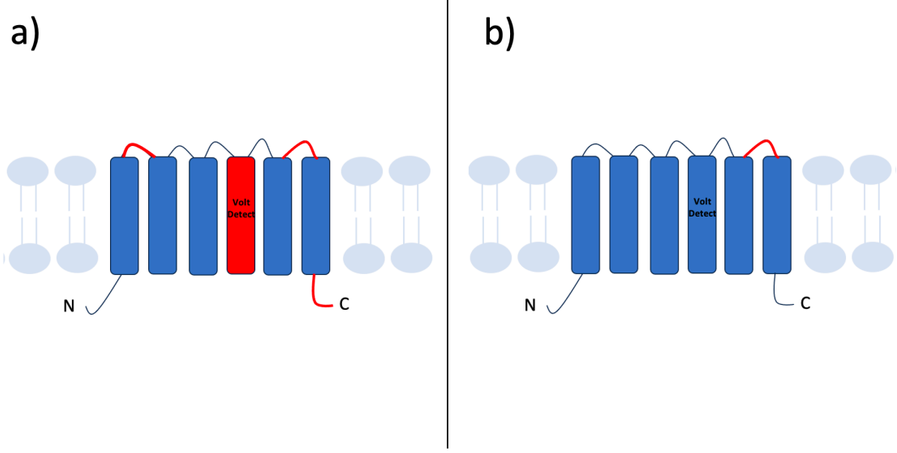

Figure 2: Sites for mutations on the voltage-gated potassium channel subunit that can lead to benign familial neonatal seizures (a) for 7.2 receptors. (b) for 7.3 receptors.

Once, the pharmacological interventions that can directly target the functions of Kv7 channels were viewed as a promising candidate to effectively treat the patients. As an adjunctive treatment, retigabine was often used for BFNS (Spoto et al., 2021). Its mechanism of action directly interacts with voltage-gated Kv7 channels (Maljevic & Lerche, 2014). Specifically, retigabine opens Kv7.2 and Kv7.3 channels and facilitates the establishment of M-current (Maljevic & Lerche, 2014). It accomplishes this through the interaction at the S5 and S6 segments of the channels, allowing them to be kept at their open states (Maljevic & Lerche, 2014). This drug thus prevents seizures by eliciting M-currents to trigger the hyper-polarizations (Maljevic & Lerche, 2014). Despite being the unique "Kv7 channel opener" (Maljevic & Lerche, 2014), the drug was unfortunately withdrawn from production in 2016 and clinical implementation in 2017 by its manufacturer company, GlaxoSmithKline (Brickel et al., 2020). This decision was made by the company since the drug was not clinically utilized very often (Brickel et al., 2020). The complications involving skin discoloration and optical pigmentation contributed to limiting the clinical use of retigabine (Brickel et al., 2020).

On the other hand, phenobarbital is administered for neonatal seizures, including BFNS (Spoto et al., 2021), as a traditional first-line treatment even though it has about 50% efficacy (Slaughter et al., 2013). Phenobarbital, as a barbiturate, facilitates the transport of chloride ions across the membrane via GABAA receptors, causing hyper-polarizations to suppress electrical excitability in the brain (Nobay & Acquisto, 2023). However, a study ironically discovered that phenobarbital may worsen the occurrence of seizures (Maeda et al., 2014). Maeda et al (18) explained that as there is a small number of potassium-chloride cotransporters present in neonatal brains, there is usually a natural build-up of intracellular chloride ions. Because barbiturates allow the extracellular flow of these chloride ions, they can produce depolarization (Maeda et al., 2014). In other words, this drug can amplify the firing of action potentials, which cause seizures.

Over the last 10 years, levetiracetam has become more commonly utilized as a first-line treatment to intervene in neonatal seizures (Spoto et al., 2021). The increase in clinical use is due to the better pharmacokinetics relative to other drugs (e.g. bioavailability over 95% and faster onset of peak concentrations) (Mruk et al., 2015). Moreover, the drug is useful in that mitigates the release of excitatory neurotransmitters from presynaptic vesicles; its mechanism of action does not involve the change of chloride ion gradient in neurons (Mruk et al., 2015). Thus, unlike phenobarbital, the generation of paradoxical depolarization is not expected in the use of levetiracetam.

Outside of the pharmacological interventions, Maljevic & Lerche (2014) suggested that the advancement of gene therapy could be beneficial as the potential treatment for BFNS. For example, Maljevic & Lerche (2014) described that previous in vivo studies noted the suppression of electrical excitability, after the viral insertion of the light-activated opsin proteins to the animal models. They explained that the stimulated halorhodopsins decreased the level of repetitive action potentials via the increased conductance of chlorine ions (Maljevic & Lerche, 2014). Maljevic & Lerche (2014) also suggested that the Kv7 receptors could be a great target for future studies on the viral interventions for BFNS.

CONCLUSIONS

The autosomal dominant mutations in KCNQ2A and KCNQ3A genes can cause defects in various segments of Kv7.2 and Kv7.3 channels. These mutant channels in the brain membrane lead to BFNS by generating smaller conductance of potassium ions, thereby making the brain membrane more prone to action potential firing. These mutations in Kv channels are known to be the only channels associated with BFNS, yet this could be due to the rare incidence reports that limits the understanding of BFNS. The mutation in the SCN2A gene is associated with the familial neonatal-infantile seizures, which happen in later onset than BFNS. Future research on other types of ion channels or environmental sources may further the knowledge of BFNS to find more optimal therapeutic interventions.

Generally, many drugs that are used for neonatal seizures are found to not be efficacious, due to the paucity of clinical drug research conducted on newborn subjects. Although retigabine was the only drug that directly opened the closed states of Kv7.2 and Kv7.3 receptors for BFNS, it is now discontinued from the market due to its harmful side effects and scarcity in clinical use (Brickel et al., 2020). Nowadays, the first-line therapeutics for BFNS include phenobarbital and levetiracetam. The former drug generates an influx of chloride ions via activation of GABAA receptors, causing hyperpolarization to slow down the repetitive firings. However, as the receptor expressions in neonatal brains are different from those of adult brains, phenobarbital can exacerbate seizures by causing an efflux of chloride ions and hence depolarization. On the other hand, levetiracetam has increasingly been used clinically, as it can prevent seizures by decreasing the discharge of excitatory neurotransmitters into the synaptic cleft. This mechanism of action by levetiracetam is favorable, as it does not involve changing the flow of the chloride ions that may trigger depolarizations, as found in the use of phenobarbital. Interestingly, a study on gene therapy was also recommended by scientists (Maljevic & Lerche, 2014) to discover a novel treatment for BFNS patients. Therefore, further research on both the pathology of BFNS and more effective pharmacological treatments is suggested.

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CONFLICT OF INTERESTS

The author declares no conflict of interest.

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The significance of sex and gender in clinical pharmacokinetics

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Abstract The consequences of the historical exclusion of cisgender women and pregnant people from pharmacological clinical trials have resulted in a dangerously low quality of care for these populations. Analyzing and interpreting significant sex- and gender-related differences in drug absorption, distribution, metabolism, and excretion are critical for adjusting dosing regimens and therapeutic drug monitoring. A deep understanding of the impact of these differences is required for practitioners and clinical researchers. This review summarizes the physiological differences between the sexes as they relate to cardiovascular, respiratory, gastrointestinal, and renal variations. A direct examination of the possible effects of these difference on the pharmacokinetics of drugs critically highlights the current knowledge gap. The importance of including all sexes in clinical trials and pharmacokinetic studies is emphasized in this review. Current and future scientists should strive toward reducing health care disparities based on sex and gender in their studies, incorporating sex and gender-specific analysis whenever possible.

INTRODUCTION

It is hardly surprising that sex differences impact the experience and symptomology of diseases. Nevertheless, sex-based biological differences and their influence on drug metabolism are rarely considered when prescribing medications and developing treatment plans (Kim et al., 2010). Based on many existing reports, cisgender women (cis women) experience nearly twice the rate of side effects than cisgender men (cis men) and represent 60% of adverse drug event-related visits to the emergency department (Budnitz et al., 2006). In 1998, the Food and Drug Administration (FDA) mandated that new drug applications must conduct sex-based studies on safety and efficacy. While this was a necessary change, most of the currently available prescription drugs received their approval from the FDA prior to 1993, without sufficient representation of men and women in their clinical trials (Zucker & Prendergast, 2020). Moreover, a 2018 meta-analysis of 107 trials controlled by the National Institutes of Health (NIH) that included cis men and women found that 72% did not perform sex-specific analysis (Zucker & Prendergast, 2020). While cis women are now participating in clinical trials at a higher rate than ever before, the ability to generalize these results is limited by a lack of sex-based analyses on physiological differences, side-effect profiles, and drug efficacy (Soldin & Mattison, 2009).

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This review aims to highlight the importance of sex-based analysis in pharmaceutical clinical trials by focusing on the influence of sex on pharmacokinetics: the study of drug absorption, distribution, metabolism, and excretion. The following section describes how, menstruation, pregnancy, and menopause cause variations in human physiology (such as cardiovascular, gastrointestinal, etc.) and could potentially explain the pharmacokinetic differences. Recent literature has begun to explore this theory, however, the capacity to spot these discrepancies has been compromised by the historical exclusion of all populations other than cis men from clinical research studies. Thus, we need to dedicate a tremendous amount of effort to correct previous non-inclusive data. This review should be used as an example to illustrate the importance of including all sexes in scientific data and to remind future scientists to minimize sex-based gaps in their research.

In addition to sex, gender can also influence drug safety and effectiveness. Gender is an individual's internal sense and experience of being male, female, both, or neither, and refers

to the characterizations that are socially constructed (World Health Organization, n.d.). Sex encompasses the biological factors associated with a person's categorization as a male, female, or intersex, and may or may not be aligned with their gender. Throughout this review, the terms female and male will be used to attribute to sex and gender non-conforming will be used as an umbrella term for adults whose gender identity differs from their sex assigned at birth. Pharmacokinetic research on the gender non-conforming population is insufficient, and the extent of generalizability of drug safety from research involving cisgender people to gender non-conforming and intersex populations is unclear (Cirrincione & Huang, 2021). In addition, gender non-conforming adults may elect to do hormone therapy (HT), gonadectomy, mastectomy, and other forms of gender-affirming procedures. While this can result in physiological and hormonal changes, their influence on the disposition of other prescribed medications is poorly understood and research studies are scarce (Cirrincione & Huang, 2021; Yager & Anderson, 2020). Given the population's disproportionate HIV burden, several phase III clinical trials for HIV pre-exposure prophylaxis (PrEP) have prioritized the inclusion of gender non-conforming people, but often lack sub studies on PrEP-HT interactions (Cirrincione et al., 2023). The FDA has failed to shed light on the critical issue of trans and intersex inclusion in their "Evaluation of Sex-Specific Data in Medical Device Clinical Studies" draft (Center for Devices and Radiological Health, 2014). One small but promising success story is the phase III clinical trial for HIV PrEP that is prioritizing the inclusion of transgender people (Cirrincione et al., 2023). While stressing the importance of further research focused on gender non-conforming and intersex populations, this review will focus on summarizing physiological and pharmacokinetic differences of sex during the menstrual cycle, pregnancy, and menopause.

DISCUSSION/LITERATURE REVIEW

Hormonal variation within the menstrual cycle, pregnancy, and menopause

Pharmacokinetics can be considerably influenced by factors such as genetics, diet, age, and lifestyle; however, this review will specifically explore how the menstrual cycle, pregnancy, and menopause affect drug pharmacokinetics (Jamei et al., 2009). In order to evaluate this effect, it is important to understand the influence of each condition in the absence of pharmacological intervention. The following are some of the highlighted factors of these complex phenomena.

The menstrual cycle is commonly divided into three phases: follicular, ovulatory, and luteal. Plasma hormone concentrations, including estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), respectively, vary significantly between different phases. During the early days of the follicular phase (known as menses), estrogen, progesterone, FSH, and LH concentrations are at a minimum (Oertelt-Prigione, 2012). In the remainder of the follicular phase, estrogen is secreted by the ovaries and will reach maximum concentration during the mid to late follicular phase. This triggers peak concentrations and subsequent ovulation (Oertelt-Prigione, 2012). During the early days of the luteal phase, the dominant follicle that released the matured egg transforms into the corpus luteum, and estrogen concentration decreases as progesterone secretion increases. Estrogen concentration peaks during the mid-luteal phase as progesterone concentration plateaus (Thiyagarajan et al., 2021). Close to the end of the luteal phase, both estrogen and progesterone concentrations decrease while FSH secretions increase (Oertelt-Prigione, 2012).

During the first 10 weeks of gestation, estrogen and progesterone are largely produced by the corpus luteum (Csapo et al., 1973). However, as the placenta develops, the corpus luteum degenerates and the placenta mainly contributes to the production of these essential hormones. Estrogen levels skyrocket throughout the pregnancy, increasing 30-fold by the time of childbirth (Betts et al., n.d.). During the 32nd week, a gradual increase of progesterone production by the placenta is observable (Rothchild, 1983). Progesterone decreases during late gestation, allowing for uterus contractions and eventually labor. Some of the other influential hormones that increase during pregnancy and require close observation are prolactin, relaxin, human chorionic gonadotropin (HCG), and human placental lactogen (hPL) (Kumar & Magon, 2012).

Menopause occurs slowly and at an average age of 51 years in females (Al-Azzawi & Palacios, 2009). Menopause is accompanied by a gradual decline in estrogen and consequently an increase in FSH. In the absence of ovulation, progesterone is only found at basal levels and secreted by the adrenal glands due to the lack of corpus luteum synthesis in menopausal people (Al-Azzawi & Palacios, 2009).

Physiological differences between sexes

Cardiovascular variations across menstrual cycle phases have been consistently documented. Those who menstruate have higher heart rates, lower diastolic blood pressure, and higher systolic blood pressure during the luteal phase (Kharitonov et al., 1994). While these factors could influence pharmacokinetics, more research is needed to determine their clinical relevance (Woodward, 2019). Gastrointestinal (GI) motility can be a rate-limiting factor for pharmacokinetics and is coordinated by the central nervous system and multiple hormones (Kashuba & Nafziger, 1998). Some researchers suggest that endogenous sex hormones can lead to slower gastric emptying in females (Hutson et al., 1989). Gastrointestinal sex differences influence all orally administered medication by impacting drug absorption and bioavailability. For example, progesterone can induce relaxation on the intestinal smooth muscles, increasing gastric emptying time (Kashuba & Nafziger, 1998).

Research focused on the menstrual cycle found that higher kidney secretions (renin activity) along with certain plasma concentrations (vasopressin and aldosterone) are higher in the luteal phase than in the follicular phase of the menstrual cycle (Forsling et al., 1981). In addition, the increase of estrogen during ovulation correlates with decreased sodium excretion (Parboosingh et al., 1974). These factors could potentially have a significant influence on the distribution and excretion processes of pharmacokinetics (Kashuba & Nafziger, 1998). Fluctuations in body weight, body temperature, and electrolytes during different phases of the menstrual cycle have been well established and can influence pharmacokinetics at different rates (Dadlani et al.,1982).

The influence of sex on pharmacokinetics

Absorption is the first step in pharmacokinetics. It is the movement of drug from the administration site into the bloodstream. This step is bypassed when the drug is administered through intravenous (IV) injection. Several factors can influence a drug's absorption rate, including gastric acid secretion, gastric emptying time, gastrointestinal surface area, blood flow, and presystemic hepatic and gut metabolism and transport (Martinez & Amidon, 2002). The influence of sex hormones on GI motility implies that there are variations in drug absorption between menopausal, pregnant, and premenopausal persons. Gastric emptying time differences have been consistently observed during pregnancy and are slower in females than males in general (Hutson et al., 1989; Singer & Brandt, 1991).

The GI enzymes responsible for drug metabolism are also influenced by sex (Freire et al., 2011). A commonly studied enzyme with significant variation between sexes is gastric alcohol dehydrogenase. Cis men typically have a larger quantity of this enzyme, leading to lower blood concentrations of ethanol after consuming the same amount of alcohol as cis women (Frezza et al., 1990). The peak of this sex-based difference in blood alcohol concentration has been found in the premenstrual period (days 21-28) rather than during menstruation (days 1-3) and intermenstrual periods (days 13-18) (Jones & Jones, 1976). Pharmacokinetics research has revealed significant sex differences in the absorption and bioavailability of certain drugs. One example of this is verapamil, a calcium channel blocker primarily used for high blood pressure (Singh et al., 1978). When administered orally, this drug is cleared more slowly in cis women than in cis men, but this trend is not observed following IV administration (Krecic-Shepard et al., 2000). These differences suggest that the underlying variation is due to sex differences in intestinal absorption processes.

The second step of pharmacokinetics is distribution, the process by which the drug leaves the bloodstream, moves between body compartments, and typically reaches the receptors. The distribution of a drug is affected by many factors, including the extent of reversible binding between a drug and plasma proteins, body mass and composition, organ blood flow, and plasma volume (Spaanderman et al., 2000). These factors are greatly influenced by sex, with sex hormone concentrations affecting the major plasma proteins responsible for drug binding. Variations in the levels of plasma binding proteins can alter the level of free (unbound) drug. The free fraction is the pharmacologically active form of the drug and it is often the primary focus during therapeutic drug monitoring studies, thus emphasizing the need for further investigation of sex-based differences in protein binding (Anderson, 2005). Alpha-1 acid glycoprotein (AAG), albumin, and alpha-globulins are three of the main plasma binding proteins for drugs. Research shows that AAG levels decrease with endogenous and exogenous estrogen interactions (Brinkman-Van der Linden et al., 1996; Succari et al., 1990; Walle et al., 1994). Some studies suggest that AAG levels may decrease over the course of pregnancy (Aquirre et al., 1998; Wood & Wood 1981), while another study suggests no observable changes in AAG concentration (Chu et al., 1981). A significant decrease in albumin has been consistently documented in pregnant people, reaching 70-80% of baseline levels by the time of delivery (Dean et al., 1980). Moreover, on average, females have more body fat and lower body weights, while males have larger average plasma volumes and higher organ blood flow, demonstrating a clear sex difference in the volume of distribution for various drugs (Nicolson et al., 2010). Volume of distribution (Vd) relates the amount of the drug that was administered and the amount measured in the blood (Smith et al., 2015). Difference in average body fat percentages between the sexes influences the volume of distribution of lipophilic (Greek word for fat loving) versus hydrophilic (Greek word for water loving) drug compounds in cis men and women. Considering the higher average body fat percentage in cis women, there would be a greater volume of distribution for lipophilic drug agents compared to hydrophilic ones (Gandhi et al., 2004). This difference has been demonstrated with lipophilic paralyzing agents such as vecuronium and rocuronium (Takaya et al., 2001; Xue et al., 1997). These drugs often have been found to have a faster onset and longer duration of action in women compared to men (Semple et al., 1994).

The third step of pharmacokinetics is metabolism, the conversion of the original drug to one or more metabolites. Hepatic (liver) clearance is influenced by both hepatic blood flow and hepatic enzymes. Although hepatic blood flow is lower in females than males, hepatic enzymes are the primary determinant underlying sex-based differences in drug metabolism (Gandhi et al., 2004). Two major enzyme-catalyzed processes drive metabolism: phase I includes oxidation, reduction, and hydrolysis, and phase II involves the conjugation of hydrophilic groups with the parent drug or its phase I metabolite. These processes increase the polarity of the drugs to increase their water solubility. Cytochrome P450 enzymes are the key components running phase I drug metabolism and have been studied extensively. The cytochrome P4503A (CYP3A) subfamily is responsible for the initial steps of the hepatic metabolism for most prescription drugs. A prominent member of this subfamily in humans is CYP3A4 (Shimada et al., 1994). Several pharmacokinetic studies revealed that males and females have significant differences in hepatic enzyme expression, where CYP3A4 levels in females is twice as high as in males (Wolbold et al., 2003). These differences stem from endogenous hormone production and variations during pregnancy, menopause, and the use of oral contraceptives (Kashuba & Nafziger, 1998). Research focusing on CYP3A4 metabolites determined that an increase in dose might be necessary to avoid loss of drug efficacy (Anderson, 2005). It has been shown that progesterone can both inhibit and induce hepatic enzymatic activity (Masuyama et al., 2001). On the other hand, estrogen can decrease the rate of oxidation of metabolites by inhibiting certain CYP enzymes (Waxman, 1988). The influence of hormonal fluctuations on phase II metabolism remains unclear; however, a minimal increase in conjugation activity has been documented by the estrogen component of oral contraceptives (Miners et al., 1983). An example of such differences in the metabolism of drugs is erythromycin. This well-studied drug is a substrate of CYP3A4 and clears more rapidly in women than men, aiding the discovery that there is a greater activity of the this enzyme in cis women (Austin et al., 1980). These results suggest that CYP34A metabolism differ by sex, however other pharmacokinetic factors may lead to varying results in overall drug clearance (Gandhi et al., 2004).

The last step of pharmacokinetics is excretion, the irreversible process of removal of drugs from the body. This process is mediated by the kidneys (renally) or the liver (via the biliary system). The renal clearance of drugs that are not actively reabsorbed or secreted into bile ducts depends on the glomerular filtration rate (GFR). GFR is proportional to body weight and thus is higher in males on average (Hermann et al., 2003). During pregnancy, a steady increase in renal blood flow and an approximately 50% increase in GRF is observed, followed by a

decrease during the last three weeks of the third trimester (Davison & Dunlop, 1980). These changes have been proposed as a possible explanation for more common adverse events in pregnant people. Due to a decreased rate of drug clearance, the same dose can result in higher drug concentrations and more frequent overdoses (Soldin & Mattison, 2009).

CONCLUSIONS

This review has documented the influences of sex-based physiological and their correlation with different pharmacokinetic processes. For many of these sections, a lack of clinical significance and/or inconsistent results were noted. The lack of adequate sample size, minimal funding, and insufficient data analysis on cis women, intersex, and gender non-conforming adult populations make it difficult to draw concrete and generalizable conclusions (Cirrincione & Huang, 2021). The Canadian Institute of Health Research has made impressive progress by requiring all government-funded research projects to integrate sex and gender into their original study design and analysis (Health Canada, 2023). This review should be used to emphasize the importance of sex based analysis and to remind researchers in all disciplines to be mindful when reviewing commonly cited but not generalizable literature.

A lack of consistency and concrete findings were also documented concerning the influence of the menstrual cycle. There are several possible explanations for these conflicting results, the first of which is inconsistent measurements for the different phases of the cycle. While these studies use semi-consistent vocabulary, some studies assign the phases based on days since menses, while others use urinary measures of hormonal excretion. Some investigations further complicate this by dividing the menstrual cycle into 2 to 5 phases and assigning different definitions to each phase (Kashuba & Nafziger, 1998). Other limiting factors include small sample sizes and limiting clinical investigations to only one cycle. Intra-individual variability and a lack of an established baseline make it difficult to conclude any significant clinical variation. Additionally, most studies only compared values averaged over the entire follicular and luteal phases, which can mask key variations within these phases (Kashuba & Nafziger, 1998). An optimal study design should be more granular in their analysis of the menstrual cycle and include measurements and analysis for menstruation, late follicular, ovulation, and late luteal phases. The first step would be to resolve this lack of agreement in the terminology and develop a gold standard for dividing different menstrual cycle periods (Elliott-Sale et al., 2021). Such standardized categorization will also be important for further research focusing on hormone and drug interactions with individuals going through HT.

Despite 20 years of legal 'enforcement' to include cis women in clinical research, substantial knowledge gaps remain. These gaps are often left for clinicians to fill in, which leaves room for assumptions and/or personal biases that can affect patient care. Gender non-conforming, intersex, and cisgender women populations are victims of this systemic medical naivete. This gap will only be overcome through a fair reallocation of resources to allow for the necessary focus on sex- and gender-based differences in clinical research. Ideally, this research would be paired with continuing physician education on the principles of pharmacokinetics and the mechanism of action of drugs in relation to dosing regimens (Soldin & Mattison, 2009). This will require foundational changes in many different areas of academia, including, but not limited to, pharmacology, medicine, public health, etc. Institutions should view studies that do not conduct sex-specific analyses as incomplete and demand justification for narrowing the sample population to only one sex (e.g. this is justifiable in gynecology research). They should also encourage studies to analyze drug pharmacokinetics during the three trimesters of pregnancy and postpartum, and employ standard definitions and measurements for each phase of the menstrual cycle (Anderson, 2005). In order to address this crisis for existing drugs, stricter and sex-based specific guidelines should be undertaken in order to develop more accurate and consistent dosing regimens for different sexes and genders. Through these measures we will be able to provide comprehensive and evidence-based health care for the members of the population who have been overlooked and under-treated for far too long.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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