Cannabis and Endometriosis: The roles of the gut microbiota and the endocannabinoid system

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Review

Cannabis and Endometriosis: The Roles of the Gut Microbiota and the Endocannabinoid System

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Abstract: There is a growing interest in the endocannabinoid system and the gut microbiota. Both areas have been suggested to play a role in endometriosis, however this is yet to be studied comprehensively. The purpose of this narrative review is to identify the gaps in endometriosis research in the context of these two areas. It underscores the limited knowledge regarding the involvement of the endocannabinoid system and gut microbiota in endometriosis. Further research in these areas is essential to unlock the therapeutic potential of cannabis and enhance the understanding of endometriosis pathogenesis.

Keywords: endometriosis; the endocannabinoid system; CB_1; CB_2; gut microbiota

Introduction

Endometriosis is a disease defined by the presence of endometrial-like tissue outside of the uterus[1]. Endometriosis is estimated to occur in up to 10% of women and those assigned female at birth during their reproductive years [2,3], with 11% of women being diagnosed by age 44 in Australia [4].[4].[2,3], with 11% of women being diagnosed by age 44 in Australia [4].[4]. Endometriosis-related chronic pelvic pain refers to a variety of pain symptoms including dysmenorrhea (period pain), dyspareunia (pain during sexual intercourse), fatigue, dyschezia (pain on bowel motions, and dysuria (pain on urination)[5–7].[5–7]. People with endometriosis are often diagnosed with various comorbidities including irritable bowel syndrome, rheumatoid arthritis, psoriasis, anxiety, depression[8] and chronic fatigue syndrome[9]. The debilitating symptoms of endometriosis impact social activities, work and career progression, finances, academic studies, mental health, emotional health and sexual/romantic relationships, ultimately impacting quality of life [5,10–13]. Furthermore, the economic burden of endometriosis impacts the Australian economy, costing AUD$30,000 per woman per year, with the most predominant factor being lost productivity, directly correlated with pain severity. Internationally this varies depending on different national economies, from US$1459 to US$20,239 per year.

One of the known mechanisms through which endometriosis symptoms occur is a result of repetitive deposition of endometrial-like tissue and active breakdown of endometriotic lesions, which in turn leads to an inflammatory cascade alongside the development of adhesions and scar tissue, as well as other factors, causing pelvic pain.[1,14]. The inflammation-mediated pain has been correlated with high levels of cyclooxygenase-2 (Cox-2) and tumour necrosis factor alpha (TNF-α) in spinal cords and brains, alongside peripheral macrophages in a murine model[15].

Endometriosis is often characterized by the altered efficiency of progesterone and estrogen hormones, leading to progesterone resistance, as observed in people with endometriosis not responding to the use of progestins, and excess estrogen levels. This depicts an imbalance which initiates local infiltration of immune cells and inflammation[16,17]. Newly established cells resulting

from inflammation activate various pathways of cell proliferation, angiogenesis, metastasis and invasion. As estrogen and progesterone receptors are responsible for proliferation and differentiation in the endometrium, imbalances result in changes in the expression of estrogen-metabolising enzymes, promoting progesterone resistance in endometriotic lesions[18].

Current treatments for endometriosis are generally considered suboptimal by those with the disease [19,20] with concerns about lack of effectiveness and problematic side effects of many medications for pelvic pain [21]. Opioid analgesics are not recommended for chronic pelvic pain due to both a lack of efficacy and safety concerns with respect to ongoing use[22]. However, despite this they continue to be prescribed; people with endometriosis have a four times greater risk of chronic opioid use compared to those without[23], and opioids are often prescribed alongside benzodiazepines[24]. Both opioids[25] and benzodiazepines[26] present significant risk of cognitive impairment, addiction and severe withdrawal symptoms, with a combination of these drugs significantly increasing the risk of overdose[27]. Surgery is considered a viable and common effective treatment [28] but often has significant costs, long waiting times [29], and substantial recurrence rates, even with expert endometriosis surgeons[30]. Access to surgery is even more reduced in developing countries and remote and rural locations[31].

Due to these issues, novel, pain management options are considered an urgent research and clinical priority in endometriosis[32–34]. Limited access to surgery often leads to people with endometriosis employing self-management strategies. The use of cannabis, either illicitly or legally, is becoming a relatively popular self-management strategy in those with endometriosis, with substantial self-report data on reduction of symptoms [35]. This review explores the potential mechanisms of action by which cannabis may modulate endometriosis symptoms.

The growing interest in the gut microbiota and its influence on various metabolic and inflammatory diseases poses the question of gut microbiota involvement in modulation and/or pathogenesis of endometriosis. Studies have also reported interactions between gut microbes and the endocannabinoid system (ECS). Both of these aspects will be explored in this review.

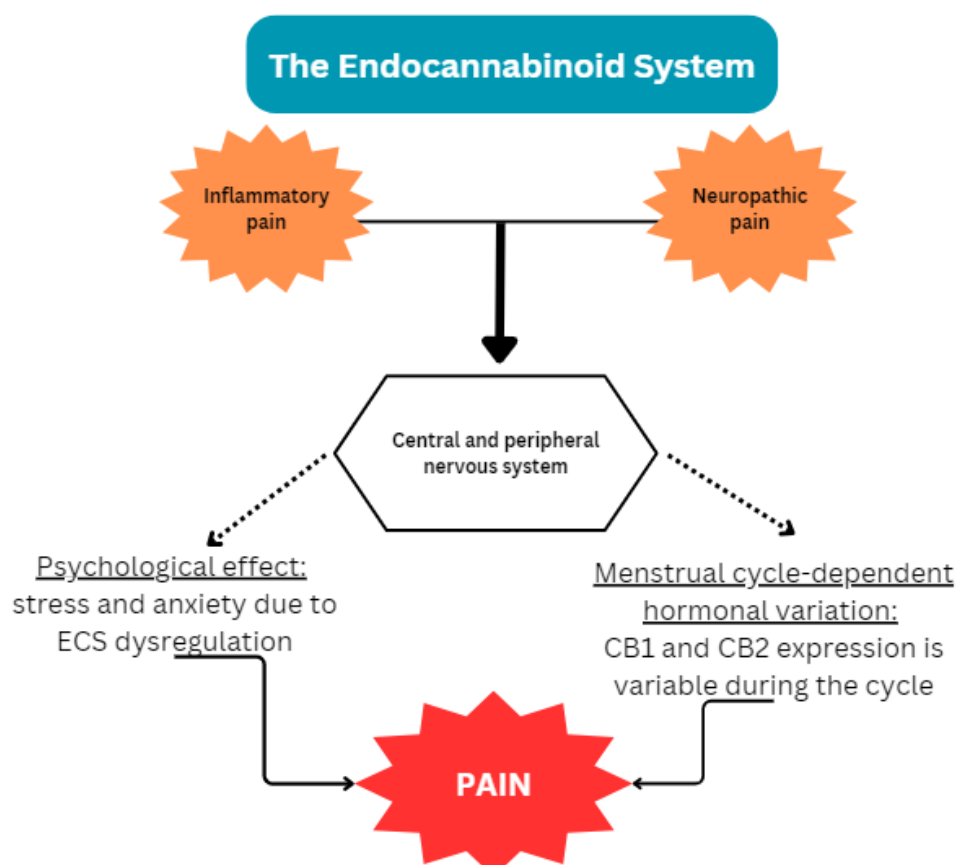*1.1. Endometriosis and the Endocannabinoid System*

Figure 1. The endocannabinoid system (ECS) in pain. As the ECS is spread throughout the central and peripheral nervous system, a psychological effect is reported as a result of endocannabinoid dysregulation. This is coupled with the menstrual-cycle dependent hormonal variation occurring due to varying levels of cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2). This figure is attributed to [36].

Research into the *Cannabis* genus during the 1990s contributed to the scientific discovery of ECS [37]. The ECS is a complex signaling system that is comprised of three major components: G-protein-coupled cannabinoid receptors (CB_1, CB_2, endocannabinoids (endogenously produced cannabinoids, including anandamide (AEA) and 2-arachidonoyl glycerol (2-AG)), ion channel transient receptor potential vanilloid 1 (TRPV1)[38] and the enzymes involved in the synthesis and catabolism of endocannabinoids [39–41].

Table 1. Key endocannabinoids and related information.

Endocannabinoid	Abbreviation	Roles
Cannabinoid receptor 1	CB_1	G-protein coupled receptor found on cells in the central and peripheral nervous system – involved in processes of mood, appetite, learning, memory and pain.
Cannabinoid receptor 2	CB_2	G-protein coupled receptor found on cells in the central and peripheral nervous system – expressed during active inflammation
Anandamide	AEA	Agonist towards CB_1 and CB_2 receptors
Arachidonoyl glycerol	2-AG	Agonist towards CB_1 and CB_2 receptors
Transient receptor potential vanilloid 1	TRPV1	Ion channel involved in endocannabinoid signalling
N-oleoylethanolamine	OEA	Fatty acid ethanolamine – involved in food intake, inflammation and pain
N-palmitoylethanolamine	PEA	Low affinity for CB_1, CB_2 and TRPV1 – induces peripheral antinociception upon activating CB_1 and CB_2
Fatty acid amide hydrolase	FAAH	Metabolises endogenous ligands
Monoglyceride lipase	MGL	Metabolises endogenous ligands

Research to date has demonstrated that the ECS is involved in homeostasis and regulation *via* neuromodulatory activity, physiological processes such as digestion [42], immune function [43], nociception [44], appetite regulation[45], cardiovascular and respiratory function[46] and sleep wake cycles[47].

The ECS influences pain modulation, making it a potential target for the treatment of chronic pain conditions such as endometriosis (Figure 1). Activation of CB_1 and CB_2 receptors by endocannabinoids or exogenous cannabinoids can suppress nociceptive processing and induce analgesia[48–51]. Similar observations were made in preclinical studies showing the analgesic effects of cannabinoid agonists on neuropathic pain[52–54]. However, clinical trials have presented mixed results in terms of the efficacy of cannabinoids in pain modulation. In a prospective randomized placebo-controlled trial, smoked cannabis (3.56% THC – participants smoked three cigarettes daily over a 4-day period) reduced daily pain experienced by adults with HIV-associated sensory neuropathy[55]. In contrast, the administration of Sativex, a low-dose THC (2.7mg/100μL) and CBD (2.5mg/100μL) combination oro-mucosal spray (used over an 8-week study period – drugs were titrated per patient) in adults with multiple sclerosis showed non-significant differences between the treatment and placebo group[56]. As studies often employ different modes of administration, which affects bioavailability, the resulting effect of cannabis varies, therefore further studies will be required to fully understand the mechanisms and therapeutic potential of the ECS.

Endocannabinoid receptors have been found throughout female reproductive tissue[41] and the use of exogenous cannabinoids may alleviate endometriosis-associated pain [57,58]. The ECS also regulates endometriosis mechanisms such as inflammation, angiogenesis, apoptosis, endometrial hyperproliferation and fibrosis [41,57–63], and this may be a potential pathway by which endometriosis lesions re-occur post surgery.

Cannabinoid receptors, specifically CB_1 receptors, are expressed on both the somata and fibres of sensory and sympathetic neurons that innervate endometriotic growths[64]. Activation of CB_1 receptors has been linked to a reduction in endometriosis-induced hyperalgesia, while blocking CB_1 receptors increases pain[65]. Moreover, elevated levels of endocannabinoids, such as AEA and 2-AG, have been observed in people with endometriosis[41]. However, the expression of CB_1 receptors in endometrial stromal cells is lower in endometriosis compared to healthy controls, suggesting a negative feedback loop that may impair the ability of endocannabinoids to control pain[66]. The levels of endocannabinoids in people with endometriosis vary across the menstrual cycle, and this is thought to be influenced by the on-demand synthesis and degradation of enzymes[67]. In the secretory phase, however, CB_1 regulation is absent, reflecting an impaired response to progesterone levels[68]. Table 1 outlines the findings of these key studies.

1.1.1. Endocannabinoid Deficiency in Endometriosis

Endometriosis has been described by some researchers as an "endocannabinoid deficiency"[68]. Plasma levels of endocannabinoid ligands AEA, 2-AG, *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) fluctuate in people with endometriosis[68]. When associating these endocannabinoids with endometriosis-associated pain severity, a correlation was noted between both increased endocannabinoid ligands in plasma and decreased local CB_1 receptor expression in people with endometriosis.[68]. These levels were studied in association with common endometriosis symptoms, where it was identified that elevated levels of AEA was linked with moderate-to-severe dysmenorrhea while elevated levels of PEA were found in people with moderate-to-severe dyspareunia[68]. This indicates a potential dysregulation in the ECS, suggesting an altered signalling process in response to endometriosis-related pain.

1.1.2. Potential Interplay of the ECS and Endometriosis

The peritoneal microenvironment is often studied in light of the establishment and progression of endometriosis. Inflammatory cytokines (interleukin-1β (IL-1β), interleukin-6 (IL-6), insulin-like growth factor (IGF-1) and tumour necrosis factor-α (TNF-α) are observed to be at a higher concentration in the peritoneal fluid of people with endometriosis [40,69–71]. The production of proinflammatory cytokines may occur as a result of disease-modified macrophages producing IGF-1, as high concentrations of IGF-1 were found in the peritoneal fluid of people with endometriosis[15]. This in turn leads to people experiencing hyperalgesia, which has been correlated with altered expression of TRPV1 in the peritoneum of people with endometriosis [72]. Levels of 2-AG and AEA have also been observed to be significantly higher in the peritoneal fluid of people with endometriosis, and this was associated with relatively higher abdominal pain[73]. The protective role of the ECS was observed in people with endometriosis, where immunohistochemistry revealed that cannabinoid agonists inhibit endometrial cell proliferation[61]. In contrast, previous studies have also shown the involvement of the ECS in endometriosis modulation and progression. ECS modulation in innervation of ectopic uterine growths was demonstrated through the abundance of CB_1 receptors on sensory and sympathetic fibres innervating ectopic growths, dorsal root ganglia and coeliac ganglia[41]. This was also observed in the epithelial cells in ovarian endometriotic lesions[74]. Such studies demonstrate the complex and contradictory role of the ECS in pain modulation, warranting more research in this area to understand the mechanisms behind the interaction of the ECS and endometriosis.

1.1.3. Exogenous cannabinoids

The intricate and multifaceted role of the ECS in pain modulation has spurred investigations into the potential impact of exogenous cannabinoids for pain management. While clinical trials are yet to be conducted, preclinical studies have explored the effects of the exogenous cannabinoid delta-9-tetrahydrocannabinol (THC) on pain management. In murine models of endometriosis, repetitive administration of botanically-derived THC demonstrated protective properties[75]. It hindered the growth of ectopic endometrial tissue while alleviating mechanical hypersensitivity in the caudal abdominal region. In an experimental mouse model varying concentrations of cannabidiol (CBD) were found to significantly reduce endometriotic implant surface area, alongside proinflammatory cytokine levels, including IL-6 and TNF-α[76]. A similar study showed that CBD administration

reduced endometriotic lesion diameter, volume and area *in vivo*, demonstrating antioxidant effects by reducing lipid peroxidation[77]. These promising findings highlight the potential therapeutic benefits of CBD and THC for endometriosis-associated pain, warranting the need for human studies.

Table 2. A summary of recent studies on endometriosis and the endocannabinoid system.

Author	Year	Type of study	Endocannabinoid/cannabinoid	Methodology	Findings
Andrieu, et al.	2022	Human – endometriosis	2-AG and AEA	LCMS analysis of endocannabinoids ELISA analysis of cytokines	An increase in abdominal pain was associated with a high level of 2-AG and a low level of AEA in the peritoneal fluid of people with endometriosis
Sanchez, et al.	2016	Human - endometriosis	CB_1	PCR analysis of menstrual cups of people with endometriosis	The availability of endocannabinoids in the human endometrium is regulated by the availability of enzymes across the menstrual cycle. The absence of CB_1 regulation in endometriosis in the secretory phase might reflect an impaired response to progesterone.
Rocha, et al.	2011	Human - endometriosis	TRPV1	Immunohistochemistry analysis of rectouterine peritoneum	There is a correlation between greater expression of TRPV1 in the peritoneum of people with endometriosis who experience chronic pelvic pain compared to those with endometriosis who do not.
Bilgic, et al.	2017	Human endometrial archive samples	Cannabinoid agonists	Immunohistochemistry analysis	Cannabinoid agonists inhibit endometriotic cell proliferation, modulating apoptosis of endometriotic cells.
Okten, et al.	2023	Female Wistar albino female rats	CBD	Immunohistochemical staining	CBD reduced endometriotic implant surface area and proinflammatory cytokine levels.
Escudero-Lara, et al.	2020	Female C57Bl/6J mice	THC	Immunostaining	Repetitive administration of THC hindered ectopic endometriotic growths, alleviating hypersensitivity.
Genovese, et al.	2022	Sprague-Dawley rats	CBD	Histological analysis ELISA	CBD reduced endometriotic lesion diameter and demonstrated antioxidant effects, as viewed by downregulated expression of MMP-9, iNOS and TGFβ).

Abbreviations: 2-AG = 2-arachidonoyl glycerol; AEA = anandamide; CB_1 = cannabinoid receptor 1; TRPV1 = transient receptor vanilloid 1; CBD = cannabidiol; THC = delta-9-tetrahydrocannabinol; LCMS = liquid chromatography-mass chromatography; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; MMP-9 = matrix metallopeptidase-9; iNOS = inducible nitric oxide synthase; TGFβ = transforming growth factor beta.

1.2. The Endocannabinoid System and the Gut Microbiota

The gut microbiota refers to the collection of trillions of microorganisms, including bacteria, archaea, viruses and fungi. The gut microbiota plays a crucial role in human health and disease, impacting immunomodulation and inflammatory processes[78].

In humans, the gut microbiota is dominated by four bacterial phyla: *Firmicutes, Bacteriodetes, Actinobacteria* and *Proteobacteria*[79]. As the phylotypic composition of these can vary amongst individuals[80], it can be implied that each host has a unique biological relationship with its gut microbiota, thereby influencing the risk of disease [81,82]. The composition of the gut microbiota in physiological processes also changes with age, implicating long-term health outcomes.

Given the nascent nature of this field of research, there is currently a scarcity of studies examining the relationship between the ECS, the gut microbiota and endometriosis. However, studies have shown the involvement of the ECS in the gut microbiota in metabolic and inflammatory disorders.

The gut is part of the ECS. CB$_1$ receptors have been localised in the gut epithelium, smooth muscle, submucosal myenteric plexus and myenteric ganglia[83,84]. CB$_2$ receptors have been detected in the plasma cells and macrophages of the gastrointestinal (GI) mucosa and submucosa, as well as intestinal epithelial cells in the GI mucosa[83,85]. While CB$_1$ receptor activation is associated with appetite regulation and relief from nausea and vomiting, CB$_2$ receptor activation mediates inflammation[86]. Endocannabinoids are known to be synthesised in various parts of the gut and their levels fluctuate, based on metabolic and inflammatory status. Endocannabinoids and exogenous cannabinoids exert opposite effects on gut permeability. For instance, when examining decreased permeability as a result of inflammation, it was demonstrated that 2-AG and AEA increased permeability, while THC and CBD decreased permeability[87,88]. Recent literature on the link between the ECS and gut microbiota is summarised in Table 2.

1.2.1. Interactions between gut microbial communities and endocannabinoids

While the interactions between gut microbiota and endocannabinoids has mainly been investigated in preclinical models, studies have linked these interactions to beneficial effects in disease states such as inflammatory bowel disorder (IBD). The administration of *Lactobacillus acidophilus* in mice led to an increase of CB$_2$ expression in intestinal epithelial cells, resulting in analgesic effects, thus decreasing visceral pain[85]. Furthermore, the administration of a probiotic mixture containing *Bifidobacteria, Lactobacilli spp.* and *Streptococcus thermophilus* in zebrafish resulted in an upregulation of CB$_1$ and CB$_2$ expression, which then led to anti-inflammatory effects[89,90].

Akkermansia muciniphila is a Gram-negative anaerobic mucus-degrading bacterium, abundantly found in healthy intestinal mucosa. *A. muciniphila* has been found to modulate gut barrier integrity. Its protective effects have been cited in relation to the ECS. A study administering *Akkermansia muciniphila* in mice fed a high-fat diet led to an increase in 2-AG, 2-OG and 2-PG levels[91]. *A. muciniphila* has been shown in regulate CB$_1$ mRNA in Caco-2 cells through increased production in outer membrane vesicles, which prevent the development of metabolic disorders such as obesity[92]. Moreover, a decrease in CB$_1$ activity resulted in reduced circulating lipopolysaccharide (LPS) levels, thus improving the inflammatory cytokine profile and intestinal permeability. This occurred alongside enhanced *A. muciniphila* and *Lachnospiraceae* levels in the gut. The gut microbiota profile of obesity is characterised by a *Firmicutes:Bacteriodetes* ratio, whereby there is an increased abundance of *Firmicutes* and a reduced abundance of *Bacteriodetes*. When chronically treating obesogenic mice with THC, this ratio was shifted and an increase in the abundance of *A. muciniphila* was observed, demonstrating the protective effects of exogenous cannabinoids [93].Further investigation is required to understand how *A. muciniphila* interacts with endocannabinoids and the outcomes of this on inflammation-associated disorders.

Studies have also examined the relationship between the ECS and gut microbial metabolites. For instance, endocannabinoids have been found to mediate the anti-inflammatory effects of SCFAs. This association was observed in an exercise intervention[94] where increase in SCFAs (including butyrate) and SCFA-producing bacteria (such as *Bifidobacterium*) were correlated with decrease in proinflammatory cytokines TNF-$\alpha$and IL-6. Furthermore, an increased abundance of SCFA-producing bacteria, such as *Bifidobacterium*, was positively associated with increases in

endocannabinoids AEA, PEA and OEA, which were correlated with CB_1 receptor levels. These studies provided an indication of how the association between ECS and the gut microbiota can be potentially utilised to improve gut inflammation.

1.2.2. The ECS-gut-brain axis

As CB_1 receptors are found in the CNS, the ECS is involved in signalling, modulating various physiologic and homeostatic processes. While the ECS-gut-brain axis has not been studied extensively, recent studies have considered the involvement of the ECS-gut-brain axis in exercise interventions, processes of food intake and disease states such as Alzheimer's disease.

While investigating the microbiota-dependent gut-brain pathway on exercise, it was shown that the CB_1-expressing TRPV1 sensory neurons are triggered by fatty acid amide metabolites from gut bacteria such as *Lachnospiraceae* and *Eubacterium* [95]. The protective effects of *Lachnospiraceae* have been well-studied in various diseases, including ulcerative colitis[96,97]. This led to analgesic effects observed after exercise, demonstrating the interaction of intestinal microbial colonization and peripheral CB_1 signaling.

Endocannabinoids such as AEA and CB_2 receptors, and enzymes such as fatty acid amide hydrolase (FAAH)   influence retrograde signalling (observed in Alzheimer's Disease) in the brain. Here, inhibitory feedback regulates neurotransmitter release[98]. Moreover, as AEA induces gut permeability, its overexpression is thought to cause a 'leaky' gut, resulting in metabolic endotoxemia[99]. As this condition leads to the release of toxins such as LPS which may cross the epithelial barrier, an inflammatory signalling pathway may develop, impacting the CNS and resulting in neuroinflammation[100].

CB_1 binding leads to increased food intake. CB_1 receptors are present in cells of the lining of the intestinal epithelium. When CB_1 receptor activity is heightened, in the small-intestinal epithelium, the release of cholecystokinin-8 (CCK-8) is inhibited, resulting in delayed satiation and overeating (in diet-induced obesity)[101], highlighting the indirect control mechanism of endocannabinoids in gut-brain neurotransmission.

1.2.3. The ECS in IBD

The use of cannabis for abdominal pain relief and other IBD symptoms is prevalent, with studies reporting use amongst 70-95% of people with IBD [86,102–104]. However, several studies have reported similar results when using endocannabinoids and/or exogenous cannabinoids in therapies for IBD. In analysis of biopsies of paediatric patients, AEA levels were found to be significantly decreased in inflamed IBD mucosa[105]. A significant increase in the expression of CB_2 receptors has also been observed in the biopsies of Crohn's disease ileum and in rectum biopsies of   ulcerative colitis, colocalised with T-lymphocyte infiltration [106] (Figure 2). Similarly, a murine IBD studies demonstrated that increasing the availability of the endogenous CB_1 and CB_2 receptor agonists, diminishes visceral pain[107–109]. Nonetheless, the issue remains that such implications have not translated to clinical trials, as noted in one study where people with IBD using cannabis reported higher incidences of abdominal pain and arthralgias[110]. Further research is required to develop insights into what may be causing these negative side effects and utilisation of the ECS as a therapy for IBD.
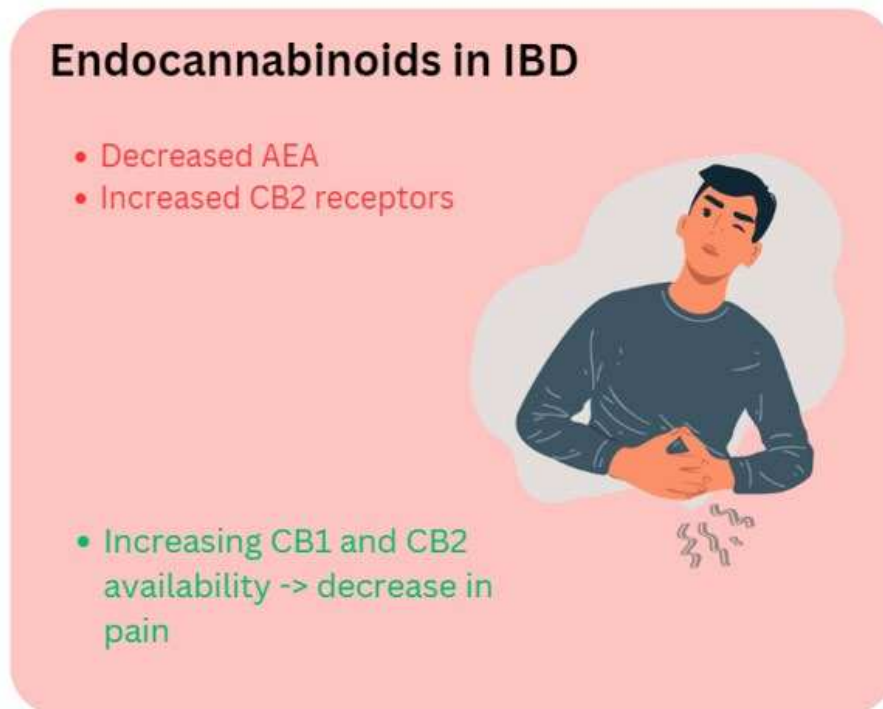
Figure 2. A decrease in AEA and increase in CB_2 receptors is reported in IBD. An increase in CB_1 and CB_2 availability leads to pain alleviation.

There is a high prevalence of inflammatory bowel syndrome (IBS) and its associated symptoms in people with endometriosis, in one study 52% (194/373) of those with endometriosis had diagnosed IBS[111], where those experiencing minimal-mild endometriosis often report more severe IBS symptoms, as compared to those with moderate-severe endometriosis. Similarly, of 160 people with diagnosed IBS, 59 had a history or recent diagnosis of endometriosis[112]. People with endometriosis are reported to have a threefold increase in likelihood of developing IBS[113]. Such studies demonstrate the importance of understanding the mechanisms through which inflammatory bowel diseases and endometriosis interact and thus the need for treatments targeting both diseases.

Table 3. A summary of recent literature on endocannabinoids and gut microbes.

Author	Year	Type of study	Endocannabinoid/cannabinoid + microbiota	Methodology	Findings
Strisciuglio, et al.	2023	Human- Crohn's disease	CB$_2$ receptors	Western blot immunofluorescence	Increased expression of CB$_2$ receptors in ileum of people with Crohn's disease.
Vijay, et al.	2021	People with knee osteoarthritis	2-AG, OEA, AEA, PEA *Bifidobacterium, Coprococcus Faecalibacterium, Colinsella*	Metabolomic analysis, Gut microbiome analysis Gene expression assay	An association between increased levels of SCFAs with circulating levels of endocannabinoids, higher microbiome diversity and low levels of proinflammatory *Colinsella.*
Pagano, et al.	2019	Pedriatic patients with ulcerative colitis and male adult CD1 mice	Cannabidivarin TRPA1	RT-PCR	In a TRPA1 antagonist manner, cannabidivarin regulates systemic inflammation and intestinal permeability.
Di Sabatino, et al.	2011	Human- Crohn's disease Mucosal samples	AEA	HPLC-MS Wound healing scratch assay Immunohistochemistry	Significantly low levels of AEA in inflamed gut mucosa.
Grill, et al.	2019	C57BL/6 mice	CB$_1$ receptors	*In situ* hybridisation Immunohistochemistry	Changes in gene expression of CB$_1$ and CB$_2$ receptors, GPR-55 and monoglycerol lipase in the gut after LPS treatment, demonstrating involvement in intestinal and systemic inflammation. These were observed in comparison to CB$_1$ and MGL knockout mice. High expression of CB$_1$ receptors in the submucosal and myenteric plexus. Reduced MGL expression in the ileum following LPS treatment. GPR-55 mRNA present alongside T-cell and macrophage markers in the ileum of healthy and treated mice.
Argueta & DiPatrizio	2017	Male C57BL/6Tac mice	CB$_1$ receptor, 2-AG and AEA	LCMS Gene expression analysis	In diet-induced obesity, the increase of CB$_1$ receptor activity inhibits CCK-8, resulting in delayed satiation and overeating.
Mehrpouya-Bahrami, et al.	2017	Male C57BL/6J mice	CB$_1$ receptor *Akkermansia muciniphila, Lachnospiraceae, Erysipelotrichaceae,*	16s RNA metagenomics	Blocking CB$_1$ receptor activity resulted in decreased LPS activity, enhancing anti-inflammatory effects by increasing the abundance of *A. muciniphia, Lachnospiraceae* and *Erysipelotrichaceae.*

Cluny, et al. 2015	Male C57BL/6N mice	*Firmicutes, Bacteriodetes A. muciniphila* THC	qPCR	Chronic administration of THC in obesogenic mice increased the *Firmicutes:Bacteriodetes* ratio and the abundance of *A. muciniphila.*
Sakin, et al. 2015	Adult male Balb-C mice and Sprague-Dawley rats	CB_1 and CB_2 receptors	Colorectal distension test Nociceptive testing	Availability of CB_1 and CB_2 receptors diminishes visceral pain.
Kiran, Rakib, Moore & Singh 2022	Female C57BL/6 mice	CB_2 inverse agonist SMM-189	Flow cytometry analysis Western blot analysis Histology	CB_2 inverse agonist SMM-189 suppressed colitis, while ameliorating the loss of body weight, reducing the inflammatory disease score and disease severity.
Dohnalova, et al. 2022	C57BL/6J mice	CB_1	Fibre photometry analysis DRG extraction, culture and calcium imaging Amplex flurometry analysis PCR, qPCR, RNA-seq Transcriptional profiling	Fatty acid amide metabolites trigger CB_1-expressing TRPV1 sensory neurons, thus elevating dopamine levels during exercise.
Jamontt, Molleman, Pertwee & Parsons 2010	Male Charles River Wister rats Distal colon tissue	CBD + THC	*In vitro* evaluation MPO assay BCA protein assay	CBD and THC reduced inflammation and functional disturbances by reducing the release of TNFa, IFNγ and nitric oxide *in vitro* and *in vivo.*
Borelli, et al. 2009	Male ICR mice	CBD	Western blot ELISA LCMS	CBD reduced colon injury, decreased expression of inflammatory markers, including nitric oxide synthase and reactive oxygen species.
Alhamoruni, et al. 2012	Caco-2 cells	CB_1 and CB_2 receptors, TRPV1, PPARγ and PPARα THC and CBD	Measurements of transepithelial electrical resistance	THC and CBD accelerated recovery of cytokine-induced intestinal permeability.
Distrutti, et al. 2014	Zebrafish	*Bifidobacteria, Lactobacilli spp.* and *Streptococcus thermophilus*	TUNEL assay	Administration of a probiotic mixture containing *Bifidobacteria, Lactobacilli spp.* and *Streptococcus thermophilus* in zebrafish led to an increase in CB_1 and CB_2 expression.
Gioacchini, et al. 2017	Adult male zebrafish	*Bifidobacteria, Lactobacilli spp.* and *S. thermophilus Bacteriodetes* and *Actinobacteria*	RT-PCR Immunohistochemistry	Administration of a probiotic mixture containing *Bifidobacteria, Lactobacilli spp.* and *S. thermophilus* in aged zebrafish resulted in an increased abundance of *Bacteriodetes* and *Actinobacteria,*

|	alongside increases in CB_1, demonstrating anti-inflammatory effects.
	Abbreviations: CB_2 = cannabinoid receptor 2; 2-AG = 2-arachidonoyl glycerol; OEA = N-oleoylethanolamine; AEA = anandamide; PEA = N-palmitoylethanolamine; TRPA-1 = transient receptor potential ankyrin type 1; CB_1 = cannabinoid receptor 1; THC = delta-9-tetrahydrocannabinol; PPARγ = peroxisome proliferator-activated receptor gamma; PPARα = peroxisome proliferator-activated receptor alpha; CBD = cannabidiol; RNA = ribonucleic acid; HPLC-MS = high protein liquid chromatography – mass spectrometry; RT-PCR = reverse transcriptase polymerase chain reaction; PCR = polymerase chain reaction; qPCR = quantitative polymerase chain reaction; LCMS = liquid chromatography mass spectrometry; TUNEL = terminal deoxylnucleotidyl transferase dUTP nick end labelling; DRG = dorsal root ganglia; RNA-seq = RNA sequencing; SCFA = short-chain fatty acid; GPR55 = G-couple protein receptor 55; MGL = monoacyl glycerol lipase (MGL); CCK-8 = cholecystokinin-8; LPS = lipopolysaccharide; IFNγ = interferon gamma; TRPV-1 = transient receptor vanilloid 1; TNF-α = tumour necrosis factor alph.1.3. Endometriosis and the Gut Microbiota.
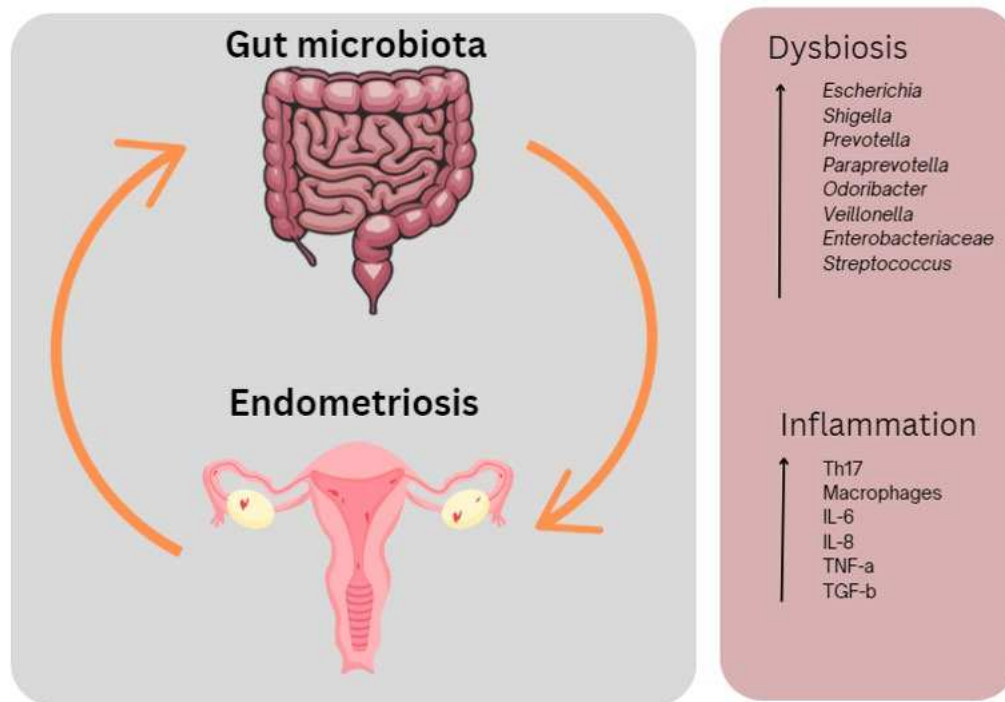
Figure 3. The interplay between the gut microbiota and endometriosis. Dysbiosis in the gut and inflammation occurring both in the gut and in the peritoneal cavity results in endometriosis-associated symptoms including pain and fatigue.

A bidirectional relationship between endometriosis and gut microbiota has been proposed (Figure 3). Current literature on the potential role of gut microbiota in endometriosis is summarised in Table 3. As gut microbes and their metabolites are involved in various immune, metabolic and epithelial functions, imbalances in the gut microbiota can trigger an inflammatory response through specific inflammatory immune cell recruitment, proinflammatory cytokine production and compromised immune surveillance. This has been observed in different disease states such as IBD [114], psoriasis[115] and cancer[116].

1.3.1. Microbial dysbiosis in endometriosis

In the endometriotic state, larger percentages of bacterial phyla *Proteobacteria, Verrucomicrobia, Streptococcus* or *Fusobacteria* have been reported. Increases in *Enterobacteriaceae, Streptococcus* and *E. coli* have been identified as dominating phyla in endometriosis cohorts[117]. Recently, *Fusobacterium* is suggested to contribute to the pathogenesis of endometriosis[118]. The dominance of *Shigella* has also been noted[119]. Such microbial communities are known to be involved in the degradation of estrogen by producing β-glucuronidase and β-glucosidase[120], resulting in the development of a high estrogen environment, promoting the progression of endometriosis[121,122].

An increase in *E. coli* is attributed as a biomarker of endometriosis and this has led to the "bacterial contamination" theory. High levels of *E. coli* have been found in faecal[123] and menstrual blood samples[124] of participants with endometriosis. *E. coli* is known to trigger TLR4 mediated growth and progression of endometriosis, resulting in pelvic inflammation[125]. This occurs through the production of LPS, triggering secretion of secondary inflammatory mediators such as NF-κB, in the peritoneal cavity, therefore, resulting in the development and progression of endometriosis.

Further research is required to unravel the intricate dynamics between the microbiota and its influence on endometriosis progression. Such investigations would not only grow the understanding of the way in which the microbiota promote endometriosis pathogenesis, but also the development of targeted therapies to improve the quality of life of those with endometriosis.

1.3.2. Microbial dysbiosis and endometriosis symptoms

Research has demonstrated correlations with specific microbes and bacterial phyla impacting symptoms in those with endometriosis. Previous studies have shown the estrogen-gut-brain axis, which is thought to influence the development of chronic stress in people with endometriosis *via* the activation of β-adrenergic signalling[126]. This has been associated with dysbiosis on a genus level, particularly decrease in *Paraprevotella, Odoribacter, Veillonella* and *Ruminococcus* [126] which are often viewed as biomarkers of endometriosis[127]. A murine study showed that a decrease in *Ruminococcus* is negatively correlated with apoptosis of endometriotic epithelial cells and increased IL-6 levels, resulting in peritoneal inflammation[128]. *Prevotella* has been found in high abundance in people with endometriosis, especially in those experiencing gastrointestinal symptoms, and is associated with constipation, bloating, flatulence, vomiting and nausea[129]. As many studies report a correlation between gut microbiota imbalances and endometriosis, continued and extensive research is essential in understanding the full potential of harnessing the gut microbiota for the treatment of endometriosis and its associated symptoms.

The vaginal microbiota is dominated by healthy *Lactobacillus*, which maintains an acidic and protective environment, preventing the growth of pathogenic bacteria. However, in endometriosis, the protective environment maintained by vaginal microbiota is impacted by an abundance of *Gardnerella, Escherichia, Shigella, Ureoplasma*[119], *Streptococcus, Moraxellae, Staphilococcus* and *Enterobacteria,* coupled with a lowered abundance of *Lactobacillus*[130]. These imbalances have been associated with endometriosis-associated pelvic pain[131,132]. An understanding of the vaginal microbiota in endometriosis, in correlation with the ECS, is required for the characterisation of treatments that may be prescribed for people with endometriosis.

Table 4. A summary of recent literature on the gut microbiota and people with endometriosis.

Author	Year	Microbiota	Methodology	Findings
Svensson, et al.	2021	*Prevotella, Bacilli, Bacteriodia, Clostridia, Coriobacteria* and *Gammaproteobacter*	16s rRNA sequencing	*Prevotella* has been associated with common endometriosis gastrointestinal symptoms such as constipation, flatulence, bloating, vomiting and nausea. People with endometriosis were found to have a high abundance of *Lactococcus (Bacilli)*, lower abundance of *Odoribacter* and higher abundance of *Prevotella*.
Sandstrom, et al.	2020	*Rumincoccus*	16s rRNA sequencing	Decrease in *Ruminococcus* correlated with an increase in IL-6 in a murine model of endometriosis, resulting in peritoneal inflammation.
Ata, et al.	2019	Complete absence of *Atopobium, Gardnerella, Streptococcus, Escherichia, Shigella, Ureoplasma*	PCR amplification 16s rRNA sequencing	Absence of *Atopobium* in vaginal and cervical microbiota Increased *Gardnerella* in cervical microbiota Dominant gut microbiota in endometriosis group – *Escherichia* and *Shigella* Predominant population of lower genital tract – *Lactobacillus Alloprevotella* significantly decreased in the cervix
Xu, et al.	2017	*Paraprevotella, Odoribacter, Veillonella* and *Ruminococcus*	16s rRNA sequencing Immunohistochemistry	The development of chronic stress in people with endometriosis occurs through the activation of β-adrenergic signalling, which occurs as a result of dysbiosis – decrease in specific unknown genus.
Khan, et al.	2010	*E. coli*	ELISA of macrophages from peritoneal fluid and epithelial/stromal cells from biopsy specimens of eutopic/ectopic endometria of women with and without endometriosis RT-PCR	Menstrual blood of people with endometriosis has a higher concentration of *E. coli* compared to healthy controls. An infiltration of macrophages in eutopic/ectopic endometria of people with endometriosis was noted.

Abbreviations: rRNA = ribosomal ribonucleic acid; ELISA = enzyme-linked immunosorbent assay; RT-PCR = reverse transcriptase polymerase chain reaction; IL-6 = interleukin-6.

6. Conclusions and Future Directions

This review summarises the relationship between endometriosis, the ECS and the gut microbiota. While studies have not completely deciphered the molecular basis of these relationships, current literature demonstrates the vital roles that the ECS and the gut microbiota play in potential implications in preventing the development of endometriosis as well as effective therapeutic strategies. The mechanisms of the ECS in both endometriosis and the gut microbiota were explored. Further research understanding the mechanisms, influence on inflammation and analysis of endocannabinoids and exogenous cannabinoids is required for the development of treatments of various diseases including endometriosis. While the impact of cannabinoids in endometriosis has been investigated, future studies are needed to comprehend how cannabinoids influence endometriosis-related pain and symptoms and how this can be implemented in the clinic, following efficacy and safety studies. This may be done through implementation of exogenous cannabinoids, which have been shown to have protective effects. An understanding of various inflammatory diseases was used to show the potential therapeutic role of the gut microbiota, while paving new avenues for further research. Finally, the relationship between the gut microbiota and endometriosis was explored, highlighting the need for further investigations of how cannabinoids may influence gut microbiota in endometriosis.

References

1. Zondervan, K.T., C.M. Becker, and S.A. Missmer, *Endometriosis.* N Engl J Med, 2020. **382**(13): p. 1244-1256.
2. Bulun, S.E., *Endometriosis.* N Engl J Med, 2009. **360**(3): p. 268-79.
3. Parazzini, F., et al., *Epidemiology of endometriosis and its comorbidities.* Eur J Obstet Gynecol Reprod Biol, 2017. **209**: p. 3-7.
4. Rowlands, I.J., et al., *Prevalence and incidence of endometriosis in Australian women: a data linkage cohort study.* BJOG, 2021. **128**(4): p. 657-665.
5. Armour, M., et al., *Endometriosis and chronic pelvic pain have similar impact on women, but time to diagnosis is decreasing: an Australian survey.* Sci Rep, 2020. **10**(1): p. 16253.
6. Armour, M., et al., *Self-management strategies amongst Australian women with endometriosis: a national online survey.* BMC Complement Altern Med, 2019. **19**(1): p. 17.
7. Tewhaiti-Smith, J., et al., *An Aotearoa New Zealand survey of the impact and diagnostic delay for endometriosis and chronic pelvic pain.* Sci Rep, 2022. **12**(1): p. 4425.
8. Lagana, A.S., et al., *Anxiety and depression in patients with endometriosis: impact and management challenges.* Int J Womens Health, 2017. **9**: p. 323-330.
9. Ramin-Wright, A., et al., *Fatigue – a symptom in endometriosis.* Human Reproduction, 2018. **33**(8): p. 1459-1465.
10. Sepulcri Rde, P. and V.F. do Amaral, *Depressive symptoms, anxiety, and quality of life in women with pelvic endometriosis.* Eur J Obstet Gynecol Reprod Biol, 2009. **142**(1): p. 53-6.
11. Armour, M., et al., *Endometriosis and the workplace: Lessons from Australia's response to COVID-19.* Aust N Z J Obstet Gynaecol, 2022. **62**(1): p. 164-167.
12. Armour, M., et al., *The cost of illness and economic burden of endometriosis and chronic pelvic pain in Australia: A national online survey.* PLoS One, 2019. **14**(10): p. e0223316.
13. Nnoaham, K.E., et al., *Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries.* Fertil Steril, 2011. **96**(2): p. 366-373 e8.
14. Cousins, F.L., et al., *New concepts on the etiology of endometriosis.* J Obstet Gynaecol Res, 2023. **49**(4): p. 1090-1105.
15. Forster, R., et al., *Macrophage-derived insulin-like growth factor-1 is a key neurotrophic and nerve-sensitizing factor in pain associated with endometriosis.* FASEB J, 2019. **33**(10): p. 11210-11222.
16. Donnez, J. and M.M. Dolmans, *Endometriosis and Medical Therapy: From Progestogens to Progesterone Resistance to GnRH Antagonists: A Review.* J Clin Med, 2021. **10**(5).
17. Yilmaz, B.D. and S.E. Bulun, *Endometriosis and nuclear receptors.* Hum Reprod Update, 2019. **25**(4): p. 473-485.
18. Donnez, J. and M.M. Dolmans, *GnRH Antagonists with or without Add-Back Therapy: A New Alternative in the Management of Endometriosis?* Int J Mol Sci, 2021. **22**(21).
19. Hawkey, A., et al., *"A day-to-day struggle": A comparative qualitative study on experiences of women with endometriosis and chronic pelvic pain.* Feminism & Psychology, 2022.
20. Evans, S., et al., *Treatment use and satisfaction in Australian women with endometriosis: A mixed-methods study.* Intern Med J, 2021.
21. Sinaii, N., et al., *Treatment utilization for endometriosis symptoms: a cross-sectional survey study of lifetime experience.* Fertil Steril, 2007. **87**(6): p. 1277-86.
22. As-Sanie, S., et al., *Healthcare utilization and cost burden among women with endometriosis by opioid prescription status in the first year after diagnosis: a retrospective claims database analysis.* J Med Econ, 2020: p. 1-7.
23. Chiuve, S.E., et al., *Chronic opioid use and complication risks in women with endometriosis: a cohort study in US administrative claims.* Pharmacoepidemiology and Drug Safety, 2021.
24. Lamvu, G., et al., *Patterns of prescription opioid use in women with endometriosis: evaluating prolonged use, daily dose, and concomitant use with benzodiazepines.* Obstetrics and gynecology, 2019. **133**(6): p. 1120.
25. Els, C., et al., *Adverse events associated with medium- and long-term use of opioids for chronic non-cancer pain: an overview of Cochrane Reviews.* Cochrane Database Syst Rev, 2017. **10**: p. CD012509.
26. Bachhuber, M.A., et al., *Increasing benzodiazepine prescriptions and overdose mortality in the United States, 1996–2013.* American journal of public health, 2016. **106**(4): p. 686-688.
27. Deering, D.E., et al., *Potential risk for fatal drug overdose perceived by people using opioid drugs.* 2018, Wiley Online Library.
28. Leonardi, M., et al., *When to Do Surgery and When Not to Do Surgery for Endometriosis: A Systematic Review and Meta-analysis.* J Minim Invasive Gynecol, 2020. **27**(2): p. 390-407 e3.
29. Armour, M., et al., *Lessons from implementing the Australian National Action Plan for Endometriosis.* Reproduction and Fertility, 2022: p. RAF-22-0003.
30. Roman, H., et al., *Long-term risk of repeated surgeries in women managed for endometriosis: a 1,092 patient-series.* Fertility and Sterility, 2023.
31. Young, K., J. Fisher, and M. Kirkman, *Endometriosis and fertility: women's accounts of healthcare.* Hum Reprod, 2016. **31**(3): p. 554-62.
32. As-Sanie, S., et al., *Assessing research gaps and unmet needs in endometriosis.* Am J Obstet Gynecol, 2019. **221**(2): p. 86-94.

33. Australian Government Department of Health. *National Action Plan for Endometriosis*. 2018   12/1/19]; Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/endometriosis

34. Armour, M., et al., *Endometriosis research priorities in Australia*. Aust N Z J Obstet Gynaecol, 2023. **n/a**(n/a).

35. Armour, M. and J. Sinclair, *Cannabis for endometriosis-related pain and symptoms: It's high time that we see this as a legitimate treatment*. Aust N Z J Obstet Gynaecol, 2023. **63**(1): p. 118-120.

36. Bouaziz, J., et al., *The Clinical Significance of Endocannabinoids in Endometriosis Pain Management*. Cannabis Cannabinoid Res, 2017. **2**(1): p. 72-80.

37. Devane, W.A., et al., *Isolation and structure of a brain constituent that binds to the cannabinoid receptor*. Science, 1992. **258**(5090): p. 1946-9.

38. Li, Y., et al., *Endocannabinoid activation of the TRPV1 ion channel is distinct from activation by capsaicin*. J Biol Chem, 2021. **297**(3): p. 101022.

39. Rodriguez de Fonseca, F., et al., *The endocannabinoid system: physiology and pharmacology*. Alcohol Alcohol, 2005. **40**(1): p. 2-14.

40. Lingegowda, H., et al., *Role of the endocannabinoid system in the pathophysiology of endometriosis and therapeutic implications*. J Cannabis Res, 2022. **4**(1): p. 54.

41. Dmitrieva, N., et al., *Endocannabinoid involvement in endometriosis*. Pain, 2010. **151**(3): p. 703-710.

42. Cuddihey, H., W.K. MacNaughton, and K.A. Sharkey, *Role of the Endocannabinoid System in the Regulation of Intestinal Homeostasis*. Cell Mol Gastroenterol Hepatol, 2022. **14**(4): p. 947-963.

43. Kienzl, M., J. Kargl, and R. Schicho, *The Immune Endocannabinoid System of the Tumor Microenvironment*. Int J Mol Sci, 2020. **21**(23).

44. Dos Santos, R.S., et al., *Cannabidiol reduces lipopolysaccharide-induced nociception via endocannabinoid system activation*. Basic Clin Pharmacol Toxicol, 2023. **133**(1): p. 16-28.

45. Rakotoarivelo, V., J. Sihag, and N. Flamand, *Role of the Endocannabinoid System in the Adipose Tissue with Focus on Energy Metabolism*. Cells, 2021. **10**(6).

46. Smith, P.J. and D.S. McQueen, *Anandamide induces cardiovascular and respiratory reflexes via vasosensory nerves in the anaesthetized rat*. Br J Pharmacol, 2001. **134**(3): p. 655-63.

47. Murillo-Rodriguez, E., et al., *The emerging role of the endocannabinoid system in the sleep-wake cycle modulation*. Cent Nerv Syst Agents Med Chem, 2011. **11**(3): p. 189-96.

48. Woodhams, S.G., et al., *The cannabinoid system and pain*. Neuropharmacology, 2017. **124**: p. 105-120.

49. Fine, P.G. and M.J. Rosenfeld, *The endocannabinoid system, cannabinoids, and pain*. Rambam Maimonides Med J, 2013. **4**(4): p. e0022.

50. Guindon, J. and A.G. Hohmann, *The endocannabinoid system and pain*. CNS Neurol Disord Drug Targets, 2009. **8**(6): p. 403-21.

51. Burston, J.J. and S.G. Woodhams, *Endocannabinoid system and pain: an introduction*. Proc Nutr Soc, 2014. **73**(1): p. 106-17.

52. Maldonado, R., J.E. Banos, and D. Cabanero, *The endocannabinoid system and neuropathic pain*. Pain, 2016. **157 Suppl 1**: p. S23-S32.

53. Curto-Reyes, V., et al., *Antinociceptive effects induced through the stimulation of spinal cannabinoid type 2 receptors in chronically inflamed mice*. Eur J Pharmacol, 2011. **668**(1-2): p. 184-9.

54. Gutierrez, T., et al., *Activation of peripheral cannabinoid CB1 and CB2 receptors suppresses the maintenance of inflammatory nociception: a comparative analysis*. Br J Pharmacol, 2007. **150**(2): p. 153-63.

55. !!! INVALID CITATION !!! (Abrams et al., 2007).

56. !!! INVALID CITATION !!! (Conte et al., 2009).

57. Di Blasio, A.M., M. Vignali, and D. Gentilini, *The endocannabinoid pathway and the female reproductive organs*. J Mol Endocrinol, 2013. **50**(1): p. R1-9.

58. Walker, O.S., A.C. Holloway, and S. Raha, *The role of the endocannabinoid system in female reproductive tissues*. J Ovarian Res, 2019. **12**(1): p. 3.

59. Clemenza, S., et al., *From pathogenesis to clinical practice: Emerging medical treatments for endometriosis*. Best Pract Res Clin Obstet Gynaecol, 2018. **51**: p. 92-101.

60. Bifulco, M., et al., *Endocannabinoids as emerging suppressors of angiogenesis and tumor invasion (review)*. Oncol Rep, 2007. **17**(4): p. 813-6.

61. Bilgic, E., et al., *Endocannabinoids modulate apoptosis in endometriosis and adenomyosis*. Acta Histochem, 2017. **119**(5): p. 523-532.

62. Gentilini, D., et al., *Endocannabinoid system regulates migration of endometrial stromal cells via cannabinoid receptor 1 through the activation of PI3K and ERK1/2 pathways*. Fertil Steril, 2010. **93**(8): p. 2588-93.

63. Leconte, M., et al., *Antiproliferative effects of cannabinoid agonists on deep infiltrating endometriosis*. Am J Pathol, 2010. **177**(6): p. 2963-70.

64. Boyd, S.T., *The endocannabinoid system*. Pharmacotherapy, 2006. **26**(12 Pt 2): p. 218S-221S.

65. Hermanson, D.J. and L.J. Marnett, *Cannabinoids, endocannabinoids, and cancer*. Cancer Metastasis Rev, 2011. **30**(3-4): p. 599-612.

66. Ayakannu, T., et al., *The endocannabinoid system and sex steroid hormone-dependent cancers.* Int J Endocrinol, 2013. **2013**: p. 259676.

67. Tanaka, K., et al., *The role of the endocannabinoid system in aetiopathogenesis of endometriosis: A potential therapeutic target.* Eur J Obstet Gynecol Reprod Biol, 2020. **244**: p. 87-94.

68. Sanchez, A.M., et al., *Elevated Systemic Levels of Endocannabinoids and Related Mediators Across the Menstrual Cycle in Women With Endometriosis.* Reprod Sci, 2016. **23**(8): p. 1071-9.

69. Sanchez, A.M., et al., *The molecular connections between the cannabinoid system and endometriosis.* Mol Hum Reprod, 2012. **18**(12): p. 563-71.

70. da Silva, C.M., et al., *Identification of local angiogenic and inflammatory markers in the menstrual blood of women with endometriosis.* Biomed Pharmacother, 2014. **68**(7): p. 899-904.

71. Naseri, S., et al., *A cross-sectional study comparing the inflammatory profile of menstrual effluent vs. peripheral blood.* Health Sci Rep, 2023. **6**(1): p. e1038.

72. Rocha, M.G., et al., *TRPV1 expression on peritoneal endometriosis foci is associated with chronic pelvic pain.* Reprod Sci, 2011. **18**(6): p. 511-5.

73. Andrieu, T., et al., *Association of endocannabinoids with pain in endometriosis.* Pain, 2022. **163**(1): p. 193-203.

74. Allam, S., et al., *Detection of Cannabinoid Receptor Expression by Endometriotic Lesions in Women with Endometriosis as an Alternative to Opioid-Based Pain Medication.* J Immunol Res, 2022. **2022**: p. 4323259.

75. Escudero-Lara, A., et al., *Disease-modifying effects of natural Delta9-tetrahydrocannabinol in endometriosis-associated pain.* Elife, 2020. **9**.

76. Okten, S.B., et al., *Cannabidiol as a potential novel treatment for endometriosis by its anti-inflammatory, antioxidative and antiangiogenic effects in an experimental rat model.* Reprod Biomed Online, 2023. **46**(5): p. 865-875.

77. Genovese, T., et al., *Molecular and Biochemical Mechanism of Cannabidiol in the Management of the Inflammatory and Oxidative Processes Associated with Endometriosis.* Int J Mol Sci, 2022. **23**(10).

78. Kinross, J.M., A.W. Darzi, and J.K. Nicholson, *Gut microbiome-host interactions in health and disease.* Genome Med, 2011. **3**(3): p. 14.

79. Ley, R.E., R. Knight, and J.I. Gordon, *The human microbiome: eliminating the biomedical/environmental dichotomy in microbial ecology.* Environ Microbiol, 2007. **9**(1): p. 3-4.

80. Costello, E.K., et al., *Bacterial community variation in human body habitats across space and time.* Science, 2009. **326**(5960): p. 1694-7.

81. Sartor, R.B., *Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics.* Gastroenterology, 2004. **126**(6): p. 1620-33.

82. Ley, R.E., D.A. Peterson, and J.I. Gordon, *Ecological and evolutionary forces shaping microbial diversity in the human intestine.* Cell, 2006. **124**(4): p. 837-48.

83. Wright, K., et al., *Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing.* Gastroenterology, 2005. **129**(2): p. 437-53.

84. Grill, M., et al., *Cellular localization and regulation of receptors and enzymes of the endocannabinoid system in intestinal and systemic inflammation.* Histochem Cell Biol, 2019. **151**(1): p. 5-20.

85. Rousseaux, C., et al., *Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors.* Nat Med, 2007. **13**(1): p. 35-7.

86. Ahmed, W. and S. Katz, *Therapeutic Use of Cannabis in Inflammatory Bowel Disease.* Gastroenterol Hepatol (N Y), 2016. **12**(11): p. 668-679.

87. Alhamoruni, A., et al., *Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability.* J Pharmacol Exp Ther, 2010. **335**(1): p. 92-102.

88. Alhamoruni, A., et al., *Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability.* Br J Pharmacol, 2012. **165**(8): p. 2598-610.

89. Gioacchini, G., G. Rossi, and O. Carnevali, *Host-probiotic interaction: new insight into the role of the endocannabinoid system by in vivo and ex vivo approaches.* Sci Rep, 2017. **7**(1): p. 1261.

90. Distrutti, E., et al., *Modulation of intestinal microbiota by the probiotic VSL#3 resets brain gene expression and ameliorates the age-related deficit in LTP.* PLoS One, 2014. **9**(9): p. e106503.

91. Everard, A., et al., *Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity.* Proc Natl Acad Sci U S A, 2013. **110**(22): p. 9066-71.

92. Ghaderi, F., et al., *Effects of active, inactive, and derivatives of Akkermansia muciniphila on the expression of the endocannabinoid system and PPARs genes.* Sci Rep, 2022. **12**(1): p. 10031.

93. Forte, N., et al., *Obesity Affects the Microbiota-Gut-Brain Axis and the Regulation Thereof by Endocannabinoids and Related Mediators.* Int J Mol Sci, 2020. **21**(5).

94. Vijay, A., et al., *The anti-inflammatory effect of bacterial short chain fatty acids is partially mediated by endocannabinoids.* Gut Microbes, 2021. **13**(1): p. 1997559.

95. Dohnalova, L., et al., *A microbiome-dependent gut-brain pathway regulates motivation for exercise.* Nature, 2022. **612**(7941): p. 739-747.

96. Frank, D.N., et al., *Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases.* Proc Natl Acad Sci U S A, 2007. **104**(34): p. 13780-5.

97. Sasaki, K., et al., *Construction of a Model Culture System of Human Colonic Microbiota to Detect Decreased Lachnospiraceae Abundance and Butyrogenesis in the Feces of Ulcerative Colitis Patients.* Biotechnol J, 2019. **14**(5): p. e1800555.

98. Castillo, P.E., et al., *Endocannabinoid signaling and synaptic function.* Neuron, 2012. **76**(1): p. 70-81.

99. Cani, P.D., et al., *Endocannabinoids--at the crossroads between the gut microbiota and host metabolism.* Nat Rev Endocrinol, 2016. **12**(3): p. 133-43.

100. Bisogno, T., A. Lauritano, and F. Piscitelli, *The Endocannabinoid System: A Bridge between Alzheimer's Disease and Gut Microbiota.* Life (Basel), 2021. **11**(9).

101. Argueta, D.A. and N.V. DiPatrizio, *Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity.* Physiol Behav, 2017. **171**: p. 32-39.

102. Actis, G.C., *The gut microbiome.* Inflamm Allergy Drug Targets, 2014. **13**(4): p. 217-23.

103. Lal, S., et al., *Cannabis use amongst patients with inflammatory bowel disease.* Eur J Gastroenterol Hepatol, 2011. **23**(10): p. 891-6.

104. Ravikoff Allegretti, J., et al., *Marijuana use patterns among patients with inflammatory bowel disease.* Inflamm Bowel Dis, 2013. **19**(13): p. 2809-14.

105. Di Sabatino, A., et al., *The endogenous cannabinoid system in the gut of patients with inflammatory bowel disease.* Mucosal Immunol, 2011. **4**(5): p. 574-83.

106. Strisciuglio, C., et al., *Increased expression of CB2 receptor in the intestinal biopsies of children with inflammatory bowel disease.* Pediatr Res, 2023. **93**(3): p. 520-525.

107. Bogale, K., et al., *Cannabis and Cannabis Derivatives for Abdominal Pain Management in Inflammatory Bowel Disease.* Med Cannabis Cannabinoids, 2021. **4**(2): p. 97-106.

108. Lichtman, A.H.N., P.S.; Booker, L.; Boger, D.L.; Cravatt, B.F., *Targeting FAAH and COX to treat visceral pain.* FASEB J, 2008. **22**(1125): p. 12.

109. Sakin, Y.S., et al., *The effect of FAAH, MAGL, and Dual FAAH/MAGL inhibition on inflammatory and colorectal distension-induced visceral pain models in Rodents.* Neurogastroenterol Motil, 2015. **27**(7): p. 936-44.

110. Coates, M.D., et al., *Symptoms and Extraintestinal Manifestations in Active Cannabis Users with Inflammatory Bowel Disease.* Cannabis Cannabinoid Res, 2022. **7**(4): p. 445-450.

111. Lee, C.E., et al., *Factors Associated with Severity of Irritable Bowel Syndrome Symptoms in Patients with Endometriosis.* J Obstet Gynaecol Can, 2018. **40**(2): p. 158-164.

112. Moore, J.S., et al., *Endometriosis in patients with irritable bowel syndrome: Specific symptomatic and demographic profile, and response to the low FODMAP diet.* Aust N Z J Obstet Gynaecol, 2017. **57**(2): p. 201-205.

113. Nabi, M.Y., et al., *Endometriosis and irritable bowel syndrome: A systematic review and meta-analyses.* Front Med (Lausanne), 2022. **9**: p. 914356.

114. Alam, M.T., et al., *Microbial imbalance in inflammatory bowel disease patients at different taxonomic levels.* Gut Pathog, 2020. **12**: p. 1.

115. Buhas, M.C., et al., *Gut Microbiota in Psoriasis.* Nutrients, 2022. **14**(14).

116. Gagniere, J., et al., *Gut microbiota imbalance and colorectal cancer.* World J Gastroenterol, 2016. **22**(2): p. 501-18.

117. Allaband, C., et al., *Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians.* Clin Gastroenterol Hepatol, 2019. **17**(2): p. 218-230.

118. Muraoka, A., et al., *Fusobacterium infection facilitates the development of endometriosis through the phenotypic transition of endometrial fibroblasts.* Sci Transl Med, 2023. **15**(700): p. eadd1531.

119. Ata, B., et al., *The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls.* Sci Rep, 2019. **9**(1): p. 2204.

120. Beaud, D., P. Tailliez, and J. Anba-Mondoloni, *Genetic characterization of the beta-glucuronidase enzyme from a human intestinal bacterium, Ruminococcus gnavus.* Microbiology (Reading), 2005. **151**(Pt 7): p. 2323-2330.

121. Kwa, M., et al., *The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer.* J Natl Cancer Inst, 2016. **108**(8).

122. Possemiers, S., et al., *The intestinal microbiome: a separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals.* Fitoterapia, 2011. **82**(1): p. 53-66.

123. Leonardi, M., et al., *Endometriosis and the microbiome: a systematic review.* BJOG, 2020. **127**(2): p. 239-249.

124. Khan, K.N., et al., *Escherichia coli contamination of menstrual blood and effect of bacterial endotoxin on endometriosis.* Fertil Steril, 2010. **94**(7): p. 2860-3 e1-3.

125. Khan, K.N., et al., *Toll-like receptor 4-mediated growth of endometriosis by human heat-shock protein 70.* Hum Reprod, 2008. **23**(10): p. 2210-9.

126. Xu, J.L., K.; Zhang, L.; Liu, Q.Y.; Huang, Y.K.; Kang, Y.; Xu, C.J. , *Dysbiosis of gut microbiota contributes to chronic stress in endometriosis patients via activating inflammatory pathway. .* Reprod Dev Med, 2017. **1**: p. 221-227.

127. Iavarone, I., et al., *Correlations between Gut Microbial Composition, Pathophysiological and Surgical Aspects in Endometriosis: A Review of the Literature.* Medicina (Kaunas), 2023. **59**(2).

128. Sandstrom, A., et al., *Effect of hysterectomy on pain in women with endometriosis: a population-based registry study.* BJOG, 2020. **127**(13): p. 1628-1635.

129. Svensson, A., et al., *Associations Between Endometriosis and Gut Microbiota.* Reprod Sci, 2021. **28**(8): p. 2367-2377.

130. Khan, K.N., et al., *Molecular detection of intrauterine microbial colonization in women with endometriosis.* Eur J Obstet Gynecol Reprod Biol, 2016. **199**: p. 69-75.
131. Chang, C.Y., et al., *A More Diverse Cervical Microbiome Associates with Better Clinical Outcomes in Patients with Endometriosis: A Pilot Study.* Biomedicines, 2022. **10**(1).
132. Salliss, M.E., et al., *The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain.* Hum Reprod Update, 2021. **28**(1): p. 92-131.