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Impact of Cannabis Exposure on Gut Barrier Function in Metabolic Health and Disease

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I dedicate this work to my wife, Viridiana, our boy Luka, and the child we are so excited to welcome into the world. Thank you for your unwavering support since my undergraduate days and for standing by me through every high and low. We did it! Without you, this would not have been possible. You have truly been my foundation and my motivation. I also dedicate this to my parents and my sisters, whose love and support have meant everything to me. Dad, thank you for all your sacrifices and the long nights you put in to make sure I had everything I needed to succeed. Mom, you have always told me, "You are meant for great things," and that belief has carried me through this journey. I hope this is one of them.

ABSTRACT OF THE DISSERTATION

Impact of Cannabis Exposure on Gut Barrier Function in Metabolic Health and Disease

by

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Doctor of Philosophy, Graduate Program in Biomedical Sciences
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Dr. Nicholas V. DiPatrizio, Chairperson

The endocannabinoid (eCB) system is a lipid-derived signaling pathway that regulates food intake, energy homeostasis, and reward, and is expressed throughout the gastrointestinal tract. In diet-induced obesity (DIO), the eCB system becomes dysregulated and may contribute to impaired gut barrier function; however, it remains unclear whether activation of this system by cannabis is protective or detrimental. Previous studies suggest that intestinal epithelial CB1 receptors (CB1Rs) regulate gut barrier integrity and inflammation, yet conflicting evidence exists regarding the effects of cannabinoid signaling on intestinal permeability.

To address this, we examined the impact of Δ^9-tetrahydrocannabinol (THC) and whole cannabis extracts on gut barrier function in male and female mice with conditional deletion of CB1Rs in the intestinal epithelium (intCB1R-/-) and control mice (intCB1R+/+). Mice were maintained on either a low-fat standard diet or a Western diet (WD) for 60 days. Intestinal permeability was assessed using 4 kDa FITC-dextran, and

mice were subsequently treated with pure Δ^9-THC or whole cannabis extract for 14 days. Intestinal tissues were collected for gene expression and lipidomic analyses.

WD-fed male mice developed obesity, whereas this effect was absent in female intCB1R-/- mice. Gut barrier dysfunction was exacerbated in male knockout mice and accompanied by increased circulating lipopolysaccharide (LPS), indicating enhanced endotoxemia. The eCB system was dysregulated in a sex- and region-specific manner. Cannabinoid treatment normalized intestinal permeability independent of epithelial CB1Rs, suggesting that CB1-independent mechanisms may contribute to barrier restoration. In male DIO mice, cannabinoids restored jejunal expression of occludin, tumor necrosis factor alpha (TNFα), and interleukin-1 beta (IL-1β). Similarly, female DIO intCB1R$^{+/+}$ mice exhibited reduced expression of occludin and IL-1β, which was restored following cannabinoid treatment. Furthermore, in males, the Western diet increased jejunal CNR2 expression, an effect reversed by exposure to cannabinoids.

These findings demonstrate that intestinal CB1Rs play a protective role in maintaining gut barrier integrity during DIO in a sex-dependent manner. Moreover, cannabis exposure can restore barrier function and inflammatory balance through both CB1-dependent and independent mechanisms, highlighting the therapeutic potential of cannabinoid signaling in metabolic disease.

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Introduction

Overview of Metabolic Disease and Diet-Induced Obesity

Metabolic disease is a growing global health burden characterized by dysregulated energy homeostasis, chronic low-grade inflammation, and an increased risk of cardiometabolic complications, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD) [1-3]. Among these conditions, obesity is a central driver, defined by excessive adiposity resulting from a sustained imbalance between caloric intake and energy expenditure [4]. The prevalence of obesity has risen dramatically in recent decades, largely driven by increased consumption of energy-dense, highly processed foods, socioeconomic factors, and reduced physical activity [5, 6].

Diet-induced obesity (DIO) is a widely used preclinical model for studying the pathophysiology of metabolic dysfunction. In both humans and animal models, Western-style diets, characterized by high fat and high sucrose, promote weight gain, adipose tissue expansion, insulin resistance, and systemic inflammation [2, 7]. Importantly, DIO recapitulates many of the metabolic and inflammatory features observed in human obesity, making it a valuable tool for mechanistic and translational research [3, 8].

A hallmark of metabolic disease is chronic, low-grade inflammation, driven by overnutrition and contributing to tissue dysfunction across multiple organ systems [1, 9]. Adipose tissue plays a central role in this process, with hypertrophic adipocytes and infiltrating immune cells producing pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β), thereby promoting systemic insulin

resistance [10-15]. In addition to adipose tissue, other organs, including the liver, skeletal muscle, and gastrointestinal tract, exhibit inflammatory and metabolic alterations during obesity [2, 16, 17].

Emerging evidence indicates that the gastrointestinal tract is a critical regulator of metabolic health. The intestine not only absorbs nutrients but also serves as a barrier that prevents the translocation of luminal contents, including microbial products, into the systemic circulation [14, 18, 19]. Disruption of this barrier during DIO has been linked to increased intestinal permeability and elevated circulating lipopolysaccharide (LPS) levels, a phenomenon termed metabolic endotoxemia [20, 21]. Elevated LPS levels can activate innate immune signaling pathways, further exacerbating systemic inflammation and metabolic dysfunction [1, 20].

Importantly, metabolic disease shows significant sex-dependent differences in both prevalence and underlying mechanisms. Clinical and preclinical studies have demonstrated that males and females differ in susceptibility to obesity, fat distribution, inflammatory responses, and metabolic outcomes [22-24]. These differences are influenced by hormonal regulation, genetic factors, and tissue-specific signaling pathways, underscoring the importance of considering sex as a biological variable in studies of metabolic disease. Emerging evidence further indicates that sex differences are not solely systemic but are also driven by cell-intrinsic mechanisms within the intestinal epithelium. Estrogen signaling, particularly through estrogen receptor alpha (ERα), has been shown to differentially regulate epithelial function, inflammatory responses, and susceptibility to intestinal injury in a sex-dependent manner [24]. For example, epithelial-specific deletion

of ERα confers protection against colonic inflammation in females, while exacerbating disease severity in males, demonstrating that identical molecular pathways can produce opposing physiological outcomes depending on sex [24]. These findings highlight a critical role for sex hormones in shaping intestinal barrier function and immune responses, providing a mechanistic basis for sex-dependent differences observed in metabolic and gastrointestinal disease.

Collectively, diet-induced obesity is a complex, multifactorial condition involving interactions among metabolic tissues, immune signaling, and intestinal physiology. Understanding the mechanisms that regulate these processes is critical for identifying therapeutic targets to mitigate obesity-associated metabolic dysfunction.

Gut Barrier Function and Intestinal Physiology

The gastrointestinal tract is central to maintaining metabolic homeostasis by regulating nutrient absorption, immune surveillance, and host-microbe interactions. Beyond its digestive functions, the intestine serves as a highly selective barrier that separates the external luminal environment from the internal environment. This barrier is essential for preventing the translocation of harmful luminal contents, including bacteria, endotoxins, and dietary antigens, into the systemic circulation [18, 19].

The intestinal barrier is composed of multiple, integrated components, including the mucus layer, epithelial lining, immune cells, and the underlying lamina propria. Among these, the intestinal epithelium serves as the primary physical barrier and consists of a single layer of polarized epithelial cells that are continuously renewed to maintain

integrity[19]. These cells are connected by specialized intercellular junctions that regulate paracellular permeability and maintain barrier function [18, 25].

Under physiological conditions, the intestinal barrier enables controlled absorption of nutrients, electrolytes, and water while restricting the passage of macromolecules and microbial products. This selective permeability is tightly regulated and modulated in response to diet, gut microbes, and host-derived factors [26, 27]. Disruption of this balance can increase intestinal permeability, often referred to as “leaky gut,” which has been implicated in a range of metabolic and inflammatory diseases [21, 27].

In the context of diet-induced obesity, growing evidence indicates that intestinal barrier dysfunction is a key contributor to metabolic dysregulation. Consumption of a Western diet has been shown to impair epithelial integrity, reduce mucus production, and disrupt tight junction organization, thereby increasing permeability [28-30]. This disruption facilitates the translocation of LPS and other bacterial components into the circulation, promoting metabolic endotoxemia and systemic inflammation [20, 28]. These processes create a cycle in which inflammation further exacerbates barrier dysfunction and metabolic impairment [31].

In addition to structural components, the intestinal barrier is influenced by interactions with the gut microbiota. Commensal bacteria help maintain the barrier by producing metabolites such as short-chain fatty acids, which support epithelial integrity and immune regulation [32, 33]. However, diet-induced changes in microbial composition, or dysbiosis, can compromise barrier function and promote inflammatory signaling [17,

29, 30]. Thus, the integrity of the intestinal barrier is shaped by interactions between body, diet, and gut microbes.

Importantly, intestinal barrier function varies along the gastrointestinal tract. Regional differences between the small intestine and colon include permeability, cellular composition, and exposure to luminal contents [19, 27]. The small intestine, particularly the jejunum, is specialized for nutrient absorption and exhibits distinct regulatory mechanisms compared to the colon, which is more heavily involved in microbial interactions and immune responses [19, 27]. Additionally, the organization of the mucus layer varies along the intestine. The colon contains a two-layered mucus structure, consisting of a dense inner layer that is firmly attached to the epithelium and limits bacterial contact, and a looser outer layer that houses commensal microbes. In contrast, the small intestine has a single, less adherent mucus layer, allowing more direct interaction between luminal contents and the epithelium[34]. These structural differences contribute to region-specific regulation of barrier function and susceptibility to disruption. These regional differences are critical when considering how metabolic disease and dietary factors affect barrier function.

Furthermore, emerging evidence suggests that intestinal barrier regulation is influenced by sex-dependent mechanisms. Differences in hormone signaling, immune responses, and microbial composition between males and females can lead to distinct patterns of barrier integrity and susceptibility to dysfunction during metabolic disease [35, 36]. In particular, estrogen signaling within intestinal epithelial cells has been shown to directly regulate epithelial function and inflammatory responses, with receptor-specific

effects producing divergent outcomes between the sexes. For example, epithelial-specific estrogen receptor signaling can drive opposing effects on intestinal inflammation and tissue repair in males and females, contributing to sex-specific differences in susceptibility to intestinal injury [24]. These observations highlight the importance of incorporating sex as a biological variable in studies examining intestinal physiology and disease.

Together, these findings highlight the intestinal barrier's role as a central regulator of metabolic health. Disruption of barrier integrity during diet-induced obesity is a critical link between the gut and systemic inflammation, providing a mechanistic framework for understanding how intestinal physiology contributes to the progression of metabolic disease.

Tight Junction Regulation in Health and Disease

The integrity of the intestinal epithelial barrier is primarily maintained by intercellular junctional complexes that regulate paracellular permeability. Among these, tight junctions are the principal regulators of selective permeability between adjacent epithelial cells [18, 25]. Tight junctions consist of transmembrane proteins, including claudins and occludin, as well as cytoplasmic scaffolding proteins such as zonula occludens-1 (ZO-1), which anchor the junctional complex to the actin cytoskeleton [18, 19]. Together, these proteins form a dynamic, tightly regulated barrier that controls the passage of ions, solutes, and macromolecules across the intestinal epithelium.

Under physiological conditions, tight junctions maintain a balance between permeability and barrier integrity, allowing selective transport while preventing uncontrolled passage of luminal contents. This regulation occurs through three distinct

permeability pathways. The “pore pathway” permits the selective passage of small ions and solutes and is largely mediated by specific claudin proteins [18, 37]. Although claudins are primarily associated with pore formation, emerging evidence suggests they can also indirectly influence the leak pathway by modulating overall tight junction structure and barrier properties [25, 37, 38]. In contrast, the “leak pathway” allows the passage of larger molecules, including macromolecules such as dextrans and bacterial products, and is more closely associated with the regulation of occludin and ZO-1 [18, 39, 40]. Disruption of this pathway is particularly relevant in metabolic disease, where increased permeability to larger solutes contributes to systemic inflammation. A third pathway, referred to as the “unrestricted pathway,” occurs under conditions of severe epithelial damage and is characterized by widespread barrier breakdown due to epithelial cell loss or apoptosis, allowing unregulated passage of luminal contents [18, 25, 40]. While this pathway is typically associated with pathological conditions such as inflammatory bowel disease, it is less prominent in the context of diet-induced obesity, which is generally characterized by more subtle alterations in tight junction regulation.

Tight junctions are highly dynamic structures that respond to a range of physiological and pathological stimuli, including dietary factors, microbial signals, and inflammatory mediators. Pro-inflammatory cytokines such as TNFα and IL-1β have been shown to disrupt tight junction organization by altering the expression, localization, and phosphorylation of junctional proteins [39, 41]. These changes can lead to redistribution of occludin and ZO-1 away from the junctional complex, thereby weakening epithelial integrity and increasing paracellular permeability [18, 39].

In diet-induced obesity, tight junction dysfunction is a key mechanism underlying impaired gut barrier function. Western diet consumption has been linked to reduced expression of tight junction proteins, altered junctional architecture, and increased intestinal permeability [28, 30, 42]. These changes are particularly evident in pathways regulating the transport of large molecules, consistent with activation of the leak pathway and increased translocation of bacterial products such as LPS [20, 28]. As a result, tight junction disruption contributes to metabolic endotoxemia and systemic inflammation, linking intestinal barrier dysfunction to the progression of metabolic disease.

Importantly, regulation of tight junction proteins exhibits both regional and sex-dependent variability. Differences in occludin and ZO-1 expression across intestinal segments and between males and females suggest that barrier integrity is modulated by local and systemic factors, including hormonal signaling and immune responses [35, 36, 43, 44]. These variations may contribute to differential susceptibility to barrier dysfunction in metabolic disease and underscore the importance of considering biological context when evaluating intestinal permeability.

Collectively, tight junctions serve as central regulators of intestinal barrier integrity, integrating signals from metabolic, immune, and microbial pathways. Disruption of tight junction structure and function is a key event in the development of obesity-associated gut barrier dysfunction and provides a mechanistic link between intestinal physiology and systemic metabolic disease.

Endocannabinoid System Biology

The endocannabinoid (eCB) system is a lipid-derived signaling network that plays a central role in regulating energy balance, metabolism, inflammation, and reward-related behaviors [45, 46]. This system consists of endogenous lipid mediators, primarily anandamide (AEA) and 2-arachidonoylglycerol (2-AG), their biosynthetic and degradative enzymes, and their primary receptors, cannabinoid receptor type 1 (CB1R) and type 2 (CB2R) [46, 47]. Together, these components form a dynamic signaling system that is widely expressed throughout the central nervous system and peripheral tissues, including the gastrointestinal tract[46, 48].

Unlike classic neurotransmitters, endocannabinoids are synthesized on demand from membrane lipids and act locally to modulate signaling[45, 47]. AEA and 2-AG bind to and activate CB1R and CB2R, G protein-coupled receptors that primarily signal through Gi/o pathways, thereby inhibiting adenylate cyclase activity and regulating downstream signaling cascades [45]. These signaling events influence a wide range of physiological processes, including appetite regulation, lipid metabolism, immune function, and gastrointestinal motility [46, 48].

CB1R is highly expressed in the central nervous system, where it plays a key role in regulating food intake and energy balance. In addition to its central functions, CB1R is expressed in peripheral tissues such as adipose tissue, liver, and the intestinal epithelium, where it contributes to metabolic regulation and inflammatory signaling [45]. CB1R activation has been linked to increased food intake, enhanced lipogenesis, and altered glucose metabolism, highlighting its role in the development of obesity and metabolic

disease[46, 49, 50]. Conversely, pharmacological blockade or genetic deletion of CB1R has been shown to improve metabolic outcomes, although systemic inhibition can produce adverse neuropsychiatric effects due to central receptor involvement [45, 51-55].

CB2R is primarily expressed in immune cells and peripheral tissues and is generally associated with modulating inflammatory responses [56]. Activation of CB2R has been shown to exert anti-inflammatory effects by reducing cytokine production and immune cell activation [48, 53-56]. Within the gastrointestinal tract, CB2R expression can be dynamically regulated in response to inflammatory stimuli, suggesting a role in maintaining immune homeostasis [48, 53, 54].

In the context of metabolic disease, the eCB system becomes dysregulated, contributing to central and peripheral alterations in energy balance and inflammation. Elevated levels of endocannabinoids, particularly 2-AG and AEA, have been observed in obesity and are associated with increased CB1R signaling activity [45, 57-59]. This dysregulation promotes metabolic disturbances, including increased adiposity, insulin resistance, and chronic inflammation [49, 60]. Importantly, alterations in eCB signaling are not uniform across tissues and may exhibit region-specific and sex-dependent patterns, particularly within the gastrointestinal tract[46, 61].

Emerging evidence suggests that the eCB system also plays a critical role in regulating intestinal physiology, including epithelial barrier function, immune responses, and interactions with the gut microbiota [42, 46, 62]. Both CB1R and CB2R are expressed in intestinal epithelial cells and in immune populations within the gut, where they modulate processes that affect barrier integrity and inflammation [48, 63]. Together, these findings

indicate that the eCB system links metabolic signaling to intestinal function by regulating barrier integrity and inflammatory responses.

Importantly, dysregulation of the eCB system during metabolic disease is not uniform across tissues but instead shows region-specific alterations within the gastrointestinal tract. For example, chronic Western diet (WD) consumption has been shown to increase levels of 2-AG and AEA in the jejunal epithelium and circulation, which contributes to enhanced peripheral CB1R signaling and promoting hyperphagia[64]. In contrast, WD feeding has been associated with reduced levels of 2-AG and related monoacylglycerols in the large intestinal epithelium, driven in part by reduced activity of the biosynthetic enzyme diacylglycerol lipase[42]. These reductions in colonic eCB tone are accompanied by increased intestinal permeability, suggesting a loss of protective eCB-mediated barrier regulation.

Together, these findings highlight a striking regional divergence in eCB signaling during diet-induced obesity. Increased eCB activity in the small intestine may promote energy intake and metabolic dysfunction, whereas decreased eCB tone in the large intestine may contribute to barrier disruption and inflammation. Whether these changes are adaptive compensatory responses or pathological drivers of metabolic stress remains unclear, but they underscore the complexity of eCB signaling in coordinating metabolic and intestinal homeostasis.

Cannabinoid Signaling in the Gastrointestinal Tract

In addition to its well-established roles in central and peripheral metabolism, the eCB system is increasingly recognized as a key regulator of gastrointestinal physiology.

Components of the eCB system, including CB1R, CB2R, and endocannabinoid ligands, are expressed throughout the gastrointestinal tract in intestinal epithelial cells, enteric neurons, and immune cells [45, 46, 48, 65]. This widespread distribution underscores the eCB system's role as an important modulator of intestinal homeostasis.

Within the gut, cannabinoid signaling helps regulate multiple processes, including motility, secretion, immune responses, and epithelial barrier function [46, 48, 66]. CB1R, in particular, is expressed in intestinal epithelial cells, where it helps maintain barrier integrity. Activation of CB1R has been shown to influence tight junction organization and paracellular permeability, suggesting a direct role in regulating epithelial barrier function [14, 15, 42, 67, 68]. In contrast, CB2R is primarily associated with immune cells in the gut and modulates inflammatory responses, which can, in turn, indirectly affect barrier integrity [56, 69].

Consistent with these roles, cannabinoid signaling has been shown to regulate intestinal permeability by modulating tight junction proteins. CB1R activation alters the expression and localization of key tight junction components, thereby influencing the passage of macromolecules across the epithelium [68, 70, 71]. Importantly, this has been directly demonstrated in vivo using conditional intestinal epithelial CB1R knockout models. Mice lacking CB1R in the intestinal epithelium exhibit exacerbated gut barrier dysfunction during Western diet–induced obesity, characterized by increased FITC-dextran permeability, elevated inflammatory gene expression, and reduced expression of tight junction proteins [42]. Together, these findings establish intestinal CB1R signaling as a protective mechanism that preserves epithelial barrier integrity under metabolic stress.

In the context of diet-induced obesity, dysregulation of the eCB system further impairs gut barrier function. High-fat or Western diets alter endocannabinoid levels and cannabinoid receptor expression in the intestine, thereby disrupting epithelial and immune signaling pathways [64, 72]. These changes are associated with increased intestinal permeability and enhanced translocation of bacterial products, such as LPS, which contribute to metabolic endotoxemia and systemic inflammation [20, 73].

Importantly, potential interactions between intestinal alkaline phosphatase (IAP) and the endocannabinoid system are beginning to emerge. Both systems are heavily engaged at the intestinal epithelium and regulate overlapping functions, including barrier integrity, inflammation, and host-microbe interactions [45]. IAP is a brush-border enzyme expressed on the apical surface of intestinal epithelial cells and plays a critical role in maintaining gut barrier integrity and mucosal homeostasis [74, 75]. IAP primarily functions by detoxifying luminal pro-inflammatory molecules, most notably LPS, by dephosphorylating the lipid A moiety, thereby reducing its ability to trigger Toll-like receptor 4 (TLR4)-mediated inflammatory signaling [75, 76].

Cannabinoid receptor signaling, particularly via CB1 receptors, has been shown to influence intestinal permeability and inflammatory responses, suggesting that eCB signaling may modulate IAP expression or activity [29, 77]. Conversely, by regulating luminal inflammatory tone and microbial composition, IAP may indirectly influence eCB system activity within the gut. Notably, human studies support this relationship, showing that obese individuals have elevated circulating levels of the endocannabinoid anandamide (AEA) and reduced duodenal expression of IAP and the tight junction protein ZO-1

compared with lean individuals [78]. Furthermore, plasma AEA levels were negatively associated with IAP and ZO-1 expression and positively associated with incretin hormone secretion, suggesting that altered endocannabinoid signaling may contribute to impaired intestinal barrier function, increased inflammation, and dysregulated metabolic signaling in human obesity [78]. Together, these observations suggest that IAP represents a complementary and potentially interacting pathway with the eCB system in regulating gut barrier function. Understanding how these systems converge may provide new insight into the mechanisms underlying metabolic disease and identify novel therapeutic targets for restoring intestinal homeostasis.

Cannabinoid signaling in the gastrointestinal tract is both region-specific and sex-dependent. Differences in cannabinoid receptor expression and endocannabinoid levels across intestinal regions, such as the jejunum and colon, suggest that the eCB system differentially regulates barrier function along the gastrointestinal tract [62, 79-81]. Additionally, sex-dependent differences in eCB signaling influence metabolic and inflammatory responses, contributing to variability in disease susceptibility and progression [61, 80]. For example, diet-induced obesity has been shown to differentially alter endocannabinoid tone between sexes, including reductions in brain AEA levels in males but not in females under high-fat diet conditions [82]. These findings highlight sex as a critical biological variable in cannabinoid-mediated regulation of gut physiology.

Collectively, these findings support a model in which the endocannabinoid system acts as a central regulator of intestinal barrier function, integrating metabolic, immune, and environmental signals. Dysregulation of this system during diet-induced obesity

contributes to barrier dysfunction by coordinating changes in tight junction organization and inflammatory signaling. These observations provide a strong rationale for investigating how intestinal cannabinoid signaling regulates gut permeability in metabolic disease.

Cannabis and Gut Barrier Function: Conflicting Evidence

Despite growing interest in the endocannabinoid system's role in gastrointestinal physiology, the impact of cannabinoid signaling on intestinal barrier function remains incompletely understood. While several studies suggest that activating cannabinoid receptors can exert protective effects on the gut, others report detrimental outcomes, highlighting a complex, context-dependent relationship between cannabinoid signaling and intestinal permeability [42, 63, 68, 77].

Under inflammatory conditions, cannabinoid signaling has often been linked to protective effects on the intestinal barrier. Phytocannabinoids such as Δ^9-THC and cannabidiol (CBD) have been shown to reduce inflammation and promote recovery of epithelial integrity, in part through CB1 receptor–dependent mechanisms. In in vitro models of cytokine-induced barrier disruption, apical application of THC or CBD accelerates recovery of transepithelial electrical resistance (TEER), indicating improved barrier function [63, 77]. These findings support a therapeutic role for phytocannabinoids in restoring epithelial integrity during inflammatory stress.

In contrast, endogenous cannabinoids can exert opposing effects under similar conditions. In the same inflammatory model, the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) further exacerbate cytokine-induced permeability in a CB1-dependent manner [63, 77]. This divergence suggests that activation of the same

receptor system can produce fundamentally different outcomes depending on the ligand, highlighting the functional complexity of the eCB system.

Additional studies demonstrate that these effects are highly dependent on both physiological context and site of action. Under hypoxic conditions, which also disrupt epithelial barrier integrity, endocannabinoids such as AEA and 2-AG increase intestinal permeability via CB1 signaling, whereas other endocannabinoid-like molecules (e.g., oleoylethanolamine and palmitoylethanolamine) can exert protective effects through alternative pathways such as TRPV1 and PPARα [83]. Moreover, the impact of cannabinoids varies depending on whether they are applied to the apical (luminal) or basolateral (systemic) surface of the epithelium, further emphasizing the importance of exposure context.

In contrast, other studies indicate that cannabinoid receptor activation may impair gut barrier function under certain conditions. Pharmacological activation of cannabinoid receptors in DIO models has been linked to increased intestinal permeability and elevated circulating LPS levels, suggesting disruption of barrier integrity [84, 85]. These findings suggest that cannabinoid signaling may have differential effects depending on the underlying metabolic or inflammatory state.

Collectively, these findings demonstrate that cannabinoid signaling does not have uniform effects on intestinal barrier function. Instead, its impact is shaped by multiple factors, including the specific ligand (endogenous vs. phytocannabinoid), receptor engagement, site of exposure, and underlying physiological or pathological conditions. In

some contexts, cannabinoid signaling promotes barrier repair and reduces inflammation, whereas in others it exacerbates permeability and inflammatory responses.

These conflicting observations highlight a critical gap in our understanding of how cannabinoid signaling regulates intestinal barrier function, particularly in the context of metabolic disease. Most studies to date have used acute inflammatory or in vitro models, with limited investigation of chronic metabolic conditions such as diet-induced obesity. Furthermore, the role of cannabinoid receptor signaling specifically within intestinal epithelial cells, as well as potential sex-dependent differences in these responses, remains poorly characterized.

Sex Differences in Metabolic and Intestinal Physiology

Metabolic disease exhibits marked sex-dependent differences in prevalence, progression, and underlying mechanisms. Clinical and preclinical studies have shown that males and females differ in susceptibility to obesity, fat distribution, insulin sensitivity, and inflammatory responses [22, 23]. These differences are shaped by hormonal, genetic, and environmental factors, with sex hormones such as estrogen playing a protective role against metabolic dysfunction in females under certain conditions [86, 87]. Consequently, male and female subjects may respond differently to dietary challenges, including Western diet exposure, and display distinct metabolic phenotypes during the development of obesity [88, 89].

In addition to systemic metabolic differences, emerging evidence indicates that intestinal physiology and barrier function are also regulated in a sex-dependent manner. Studies have shown that males and females differ in intestinal permeability, immune

responses, and susceptibility to gut barrier dysfunction [36, 90]. These differences may be mediated by sex-specific regulation of tight junction proteins, immune signaling pathways, and interactions with the gut microbiota [35, 36]. For example, sex-based differences in the expression and localization of key tight junction proteins have been reported, suggesting that barrier integrity is differentially regulated in males and females [35, 91].

Emerging evidence indicates that these sex-dependent differences are driven, in part, by intrinsic signaling mechanisms within the intestinal epithelium. Estrogen receptor alpha (ERα) signaling in intestinal epithelial cells has been shown to have opposing effects in males and females. Specifically, epithelial-specific ERα deletion protects female mice from colitis-induced intestinal injury, while exacerbating inflammation and epithelial damage in males [24]. These sex-specific outcomes are associated with differential regulation of epithelial gene expression, including tight junction proteins and pathways involved in epithelial proliferation and differentiation, highlighting a direct role for sex hormone signaling in modulating barrier integrity at the cellular level.

Sex-dependent differences also extend to inflammatory signaling pathways that regulate intestinal barrier function. Pro-inflammatory cytokines such as TNFα and IL-1β, which disrupt tight junction organization, may be differentially regulated between males and females during metabolic disease [22, 90]. These differences in inflammatory tone can contribute to variation in barrier integrity and susceptibility to metabolic endotoxemia.

Importantly, the endocannabinoid system is also subject to sex-dependent regulation. Differences in endocannabinoid levels, cannabinoid receptor expression, and downstream signaling have been observed between males and females across multiple

tissues, including the gastrointestinal tract [80, 92]. These variations may influence how cannabinoid signaling affects metabolic processes, immune responses, and intestinal barrier function. In particular, sex-dependent differences in CB1R and CB2R signaling may contribute to variability in responses to both endogenous endocannabinoids and exogenous cannabinoids, such as Δ^9-THC [80, 92].

Despite these observations, many studies investigating metabolic disease, intestinal barrier function, and cannabinoid signaling have been conducted predominantly in male models, limiting our understanding of how these processes differ between the sexes [93, 94]. This represents a significant gap in the literature, particularly given the growing recognition of sex as a critical biological variable in preclinical and clinical research.

Collectively, these findings highlight the need to account for sex-dependent mechanisms in studies of metabolic disease and intestinal physiology. Differences in metabolic regulation, epithelial barrier function, inflammatory signaling, and endocannabinoid system activity suggest that males and females may respond fundamentally differently to dietary stress and cannabinoid exposure. Therefore, including both sexes in experimental design is essential to accurately define the role of cannabinoid signaling in gut barrier function during diet-induced obesity.

Rationale and Knowledge Gaps

Collectively, the preceding sections highlight the complex interplay among metabolic disease, intestinal barrier function, and endocannabinoid signaling. Diet-induced obesity is associated with chronic, low-grade inflammation and disruption of intestinal barrier integrity, leading to increased permeability and metabolic endotoxemia [20, 21, 29].

Tight junction proteins such as occludin and ZO-1 play central roles in regulating this process, particularly through the leak pathway, which governs the passage of macromolecules across the intestinal epithelium [18, 19]. Concurrently, the endocannabinoid system has emerged as an important regulator of metabolic and gastrointestinal physiology, with roles in inflammation, epithelial function, and barrier integrity [45, 46, 62].

Despite these advances, significant gaps remain in our understanding of how cannabinoid signaling modulates intestinal barrier function in the context of metabolic disease. First, the effects of cannabinoid receptor activation on gut permeability are inconsistent across studies, with evidence supporting both protective and harmful outcomes. These discrepancies may stem from differences in experimental models, physiological context, and routes of cannabinoid exposure, but the mechanisms underlying these opposing effects remain unclear [46, 63, 68, 77].

Second, although the endocannabinoid system is expressed throughout the gastrointestinal tract, the specific role of cannabinoid receptor signaling in intestinal epithelial cells in regulating barrier integrity remains unclear. Because epithelial cells form the primary physical barrier and directly regulate paracellular permeability, understanding how epithelial cannabinoid signaling influences tight junction organization and function represents a critical gap in the field [46, 67].

Third, most studies examining cannabinoid signaling and intestinal barrier function have been conducted in lean or acute inflammatory models, with limited investigation in the context of diet-induced obesity. Because metabolic disease introduces distinct

alterations in inflammatory tone, endocannabinoid levels, and barrier function, it is essential to evaluate cannabinoid effects within a metabolically relevant framework.

Finally, sex-dependent differences in metabolic disease, intestinal physiology, and endocannabinoid signaling are increasingly recognized yet remain underexplored in studies of gut barrier function. The extent to which cannabinoid signaling differentially regulates intestinal permeability and inflammatory responses in males and females during diet-induced obesity remains poorly understood [22, 80, 92].

To address these knowledge gaps, the present study investigates the role of cannabinoid signaling in regulating intestinal barrier function during diet-induced obesity using a model of conditional deletion of CB1 receptors in the intestinal epithelium. By examining the effects of both pure Δ^9-THC and whole cannabis extracts, and assessing outcomes across sexes and intestinal regions, this work aims to clarify the mechanisms by which cannabinoid signaling influences gut barrier integrity under metabolically relevant conditions.

Hypothesis and Specific Aims

Based on the current literature and identified knowledge gaps, the central hypothesis of this study is that cannabis exposure provides protection against gut barrier dysfunction in the context of metabolic disease. Specifically, it is proposed that cannabinoid signaling modulates intestinal barrier integrity and associated inflammatory responses during diet-induced obesity.

To test this hypothesis, the following specific aims were developed:

Aim 1: To determine the impact of cannabis exposure on gut barrier function in a model of diet-induced obesity.

This aim evaluates intestinal permeability and barrier integrity following cannabinoid exposure, focusing on tight junction regulation and regional differences within the gastrointestinal tract.

Aim 2: To determine the impact of cannabis exposure on inflammatory pathways in a model of diet-induced obesity.

This aim assesses how cannabinoid exposure influences inflammatory signaling associated with barrier dysfunction, including the expression of pro-inflammatory cytokines and systemic markers of endotoxemia.

Chapter 1: Impact of Cannabis Exposure on Gut Barrier Function in Metabolic Health and Disease

Abstract

The endocannabinoid (eCB) system is a lipid-derived signaling pathway that regulates food intake, energy homeostasis, and reward and is expressed throughout the gastrointestinal tract [57]. In diet-induced obesity (DIO), the eCB system becomes dysregulated and may contribute to impaired gut barrier function; however, it remains unclear whether cannabis-induced activation of this system is protective or detrimental. Previous studies suggest that intestinal epithelial CB1 receptors (CB1Rs) regulate gut barrier integrity and inflammation [42], yet evidence on the effects of cannabinoid signaling on intestinal permeability is conflicting.

To address this, we examined the impact of Δ^9-tetrahydrocannabinol (THC) and whole cannabis extracts on gut barrier function in male and female mice with conditional deletion of CB1Rs in the intestinal epithelium (intCB1R-/-) and control mice (intCB1R+/+). Mice were maintained on either a low-fat standard diet or a Western diet (WD) for 60 days. Intestinal permeability was assessed using 4 kDa FITC-dextran, and mice were subsequently treated with 5mg/kg of pure Δ^9-THC or whole cannabis extract for 14 days. Intestinal tissues were collected for gene expression and lipidomic analyses.

WD-fed male mice developed obesity, whereas this effect was absent in female intCB1R-/- mice. Gut barrier dysfunction was exacerbated in male knockout mice and accompanied by increased circulating LPS, indicating enhanced endotoxemia. The eCB system was dysregulated in a sex- and region-specific manner. Cannabinoid treatment

normalized intestinal permeability independent of epithelial CB1Rs, suggesting that CB1-independent mechanisms may contribute to barrier restoration. In male intCB1+/+ DIO mice, cannabinoids restored jejunal expression of occludin, TNFα, and IL-1β. Similarly, female DIO intCB1R+/+ mice exhibited reduced expression occludin and IL-1β, which was restored following cannabinoid treatment. Furthermore, in males, the Western diet increased jejunal CNR2 expression, an effect reversed by exposure to cannabinoids.

These findings demonstrate that intestinal CB1Rs play a protective role in maintaining gut barrier integrity during DIO in a sex-dependent manner. Moreover, cannabis exposure can restore barrier function and inflammatory balance through both CB1-dependent and independent mechanisms, highlighting the therapeutic potential of cannabinoid signaling in metabolic disease.

Introduction

Metabolic disease is characterized by chronic, low-grade inflammation and disrupted energy homeostasis, with obesity a major contributor to comorbidities such as insulin resistance and cardiovascular disease [1-3]. Obesity is a major driver of these disorders and is strongly associated with Western-style diets rich in fat and sucrose [2, 4-8]. In addition to effects on adipose tissue, liver, and skeletal muscle, obesity alters gastrointestinal physiology, supporting the concept that the intestine is an important regulator of metabolic health [12, 13, 16, 18-21].

The intestine functions not only in nutrient absorption but also as a selective barrier that limits the translocation of luminal contents, including bacteria, endotoxins, and dietary

antigens, into the circulation [18, 19]. This barrier is maintained by multiple integrated components, including the mucus layer, intestinal epithelium, immune cells, and intercellular junctional complexes[18, 19, 25]. Among these, tight junction proteins such as occludin, zonula occludens-1 (ZO-1), and members of the claudin family are central regulators of paracellular permeability [18, 25, 37]. Disruption of tight junction organization, particularly along the leak pathway, increases the passage of macromolecules, such as bacterial products and dextrans, across the epithelium, thereby contributing to inflammation and metabolic dysfunction [18, 38-41].

Growing evidence indicates that gut barrier dysfunction is a key feature of diet-induced obesity (DIO). Consumption of Western diets impair epithelial integrity, disrupt tight junction organization, alter mucus-associated defenses, and increase intestinal permeability [20, 21, 28-31]. These effects increase translocation of LPS and other microbial products into the circulation, promoting metabolic endotoxemia and systemic inflammation [20, 21, 28]. Importantly, barrier regulation varies along the gastrointestinal tract. The small intestine, particularly the jejunum, is specialized for nutrient sensing and absorption, whereas the colon is more heavily shaped by microbial interactions and immune regulation [19, 27, 34, 95, 96]. These regional differences are likely important when evaluating how metabolic stress alters intestinal physiology.

In parallel with tight junction regulation, intestinal alkaline phosphatase (IAP) has emerged as a key modulator of gut barrier integrity. IAP is a brush-border enzyme that detoxifies luminal LPS by dephosphorylating lipid A, thereby reducing Toll-like receptor 4-mediated inflammatory signaling [74, 75, 97]. Reduced IAP activity has been linked to

increased gut permeability, endotoxemia, and metabolic dysfunction, whereas exogenous IAP improves barrier function and attenuates features of metabolic disease in preclinical models [76, 98, 99]. Notably, human studies further support this relationship, showing that obesity is associated with elevated circulating anandamide (AEA) and reduced duodenal expression of IAP and ZO-1, suggesting that altered endocannabinoid signaling may be linked to impaired barrier regulation in obesity [78].

The endocannabinoid (eCB) system is a lipid-derived signaling network that regulates energy balance, inflammation, gut-brain signaling, and intestinal physiology [45, 46, 48]. This system comprises the endogenous ligands AEA and 2-arachidonoylglycerol (2-AG), their metabolic enzymes, and the cannabinoid receptor type 1 (CB1R and type 2 (CB2R) [45-47]. Within the gastrointestinal tract, CB1R is expressed in intestinal epithelial cells, enteric neurons, and other peripheral tissues, whereas CB2R is more strongly associated with immune cell populations [48, 56, 69]. Emerging evidence indicates that cannabinoid signaling modulates intestinal permeability, inflammation, and tight junction regulation [42, 50, 54, 62, 68, 77]. Importantly, conditional deletion of CB1R in the intestinal epithelium exacerbates diet-induced gut barrier dysfunction, increasing FITC-dextran permeability, inflammatory gene expression, and loss of barrier-associated transcripts, indicating that epithelial CB1R signaling plays a protective role during metabolic stress [42].

Despite this, the effects of cannabinoid signaling on gut barrier function remain complex and, in some cases, conflicting. Under inflammatory conditions, phytocannabinoids such as Δ^9 -THC and CBD can improve epithelial barrier recovery and

reduce permeability, whereas endocannabinoids such as AEA and 2-AG may worsen permeability in the same in vitro models [63, 77]. Additionally, these studies showed that cannabinoid effects depend on physiological context, ligand identity, and site of exposure across the epithelium [63, 77, 83]. In metabolic disease, the eCB system also becomes dysregulated in a region-specific manner. Western diet feeding increases jejunal and circulating eCB tone, which is linked to hyperphagia, while reducing 2-AG and related monoacylglycerols in the large intestinal epithelium, where reduced eCB tone is associated with impaired barrier regulation [42, 64]. Together, these findings suggest that cannabinoid signaling may exert distinct effects across intestinal regions and disease states.

Sex is another important variable that may shape intestinal and cannabinoid responses. Males and females differ in susceptibility to obesity, inflammatory tone, intestinal permeability, and endocannabinoid signaling [22-24, 35, 36, 61, 80, 86-90]. Recent evidence indicates that these differences are not solely systemic but can arise from intrinsic signaling pathways within the intestinal epithelium. For example, epithelial-specific deletion of estrogen receptor alpha protects female mice from colitis-induced injury while worsening inflammation in males, demonstrating that identical epithelial pathways can produce opposite outcomes depending on sex [24]. However, sex-dependent regulation of cannabinoid signaling in the intestinal epithelium during diet-induced obesity remains poorly defined.

To address this gap, the present study examined the impact of cannabis exposure on intestinal barrier function using a model of conditional deletion of CB1 receptors in the intestinal epithelium. By evaluating the effects of both Δ^9-THC and whole cannabis

extracts across sexes and intestinal regions, this work aims to define the role of cannabinoid signaling in regulating gut barrier integrity under metabolically relevant conditions.

Materials and Methods

Ethical Approval

All procedures met the U.S. National Institutes of Health guidelines for the care and use of laboratory animals in experiments and were carried out and approved according to the guidelines by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Riverside (Animal Use Protocol #9).

Animals and Diets

Male and female 6–8-week-old C57BL/6N mice (Taconic Biosciences, Oxnard, CA, USA), intestinal epithelium–specific CB1 receptor knockout mice (intCB1R-/-) [67], and littermate control mice expressing functional CB1 receptors in the intestinal epithelium (intCB1R+/+) were housed under standard laboratory conditions with ad libitum access to water and food. Mice were fed either a standard laboratory diet (SD; Teklad 2020x, Envigo, Huntingdon, UK; 16% kcal from fat, 24% kcal from protein, 60% kcal from carbohydrates, primarily as starch) or a Western diet (WD; Research Diets D12079B, New Brunswick, NJ, USA; 40% kcal from fat, 17% kcal from protein, 43% kcal from carbohydrates, primarily as sucrose).

Generation of Intestinal Epithelium–Specific CB1R Knockout Mice

Conditional intestinal epithelium–specific CB1 receptor knockout mice (intCB1R-/-; Cnr1tm1.1mrl/vil-CreERT2) were generated as previously described by crossing Cnr1tm1.1mrl mice (Taconic Biosciences; Model #7599) with vil-CreERT2 mice

(originally provided by Randy Seeley, University of Michigan, Ann Arbor, MI, USA, with permission from Sylvie Robine, Curie Institute, Paris, France). This breeding strategy resulted in substantial knockdown of Cnr1 gene expression throughout the intestinal epithelium, including the large intestine. Control mice (intCB1R+/+) were generated as previously described and carried only the floxed Cnr1 transgene (i.e., Cnr1tm1.1mrl [67, 100] without Cre recombinase expression. All animal procedures, including housing, experimental manipulations, and euthanasia, were approved by the University of California, Riverside Institutional Animal Care and Use Committee (IACUC protocol #9). All experiments were conducted in accordance with the National Institutes of Health guidelines, the Animal Welfare Act, and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Chemical Preparation and Administration

To induce recombination, both intestinal epithelium–specific CB1 receptor knockout (intCB1R-/-) and control (intCB1R+/+) mice received intraperitoneal (IP) injections of tamoxifen at a dose of 40 mg/kg/4 ml (Sigma-Aldrich, St. Louis, MO, USA) once daily for five consecutive days, as previously described [67]. For assessment of intestinal permeability, fluorescein isothiocyanate (FITC)–conjugated dextran (4 kDa; Sigma-Aldrich, St. Louis, MO, USA) was administered by oral gavage as described below.

Drug Preparation and Administration

Following 60 days on their respective diets, mice were treated for 14 consecutive days with either Δ^9-tetrahydrocannabinol (THC; 5 mg/kg) or a whole-cannabis extract standardized to deliver an equivalent THC dose. The vehicle solution consisted of 5% ethanol, 5%

Tween-80 (Chem Implex Intl., Wood Dale, IL, USA), and 90% sterile saline (0.9% w/v; LabChem Inc., PA, USA). Drug solutions were prepared fresh daily. Tween-80 was first added to THC or cannabis extract aliquots, followed by heated water-bath sonication for 30 minutes to ensure proper emulsification. Sterile saline was then added dropwise, followed by an additional 10 minutes of sonication. All treatments were administered via intraperitoneal (IP) injection using 25-gauge needles once daily, 1 hour prior to the onset of the dark cycle (6:00 PM). Injection sites were alternated between the left and right peritoneal cavity throughout the treatment period to minimize local tissue irritation.

Cannabis Extraction and LCMS quantitation

Whole-cannabis flower was weighed, submerged in ice-cold 100% ethanol (KOPTEC, Decon Labs, PA, USA), and agitated on a platform shaker at 125 rpm for 15 minutes. Δ^9-tetrahydrocannabinol (THC) and cannabis flower (Batch/Barrel #1304-1) were generously provided by the National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH). The resulting extract was filtered and evaporated under a gentle stream of ultrapure nitrogen gas (99.9%) at 37°C. The dried extract was then heated at 120°C for 1 hour to decarboxylate acidic cannabinoid precursors. Phytocannabinoid composition, including acidic precursors such as tetrahydrocannabinolic acid (THCAA) and cannabidiolic acid (CBDA), was quantified using an Acquity Class I UPLC system coupled to a Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, MA, USA). Chromatographic separation was performed using an Acquity UPLC BEH C18 column, as previously described [64, 101]. Analytes were detected using multiple reaction monitoring (MRM) in positive electrospray ionization mode (ES+). The monitored mass transitions

(m/z), cone voltages, and collision energies were as follows: Δ⁹-THC (315.2>193.0) = 8v, 18v; THCa-a (359.3>94.9) = 2v, 26v; CBD (315.2>122.9) = 8v, 30v; Δ⁹-THC-D3 (318.3>196.1) and CBD-D3 (318.3>196.1) = 18v, 18v; CBN (311.2>195.01) = 32v, 30v; CBG (317.2>193.06) = 34v, 30v. Deuterated internal standards were used for calibration and quantification, and standard curves were generated for each analyte. Extracts were prepared in ethanol, diluted to achieve a final Δ⁹-THC dose of 5 mg/kg for in vivo administration, and stored at −20°C until use. Concentrations of individual cannabinoids in the administered extract are provided in Table 1, and representative chromatograms are shown in Supplemental Figure 1.1.

Δ^9-THC	**THCA-A**	**THCV**	**CBD**	**CBN**	**CBG**
5.0	0.121	0.063	0.03	0.559	0.171

Table 1.1: Doses of phytocannabinoids in cannabis extracts

All values represent the quantity of cannabinoids contained in the extract delivered to mice in mg per kg body weight. Δ^9THC = Δ^9-tetrahydrocannabinol; THCAA = Δ^9-tetrahydrocannabinolic acid; THCV = tetrahydrocannabivarin; CBD = cannabidiol; CBN = cannabinol; CBG = cannabigerol. (Adapted from Avalos, Olmos et. al 2026).

In Vivo Intestinal Permeability Assay and Tissue Collection

To prevent coprophagia, mice were housed in elevated wire-bottom cages for a 72-hour acclimation period before and throughout the FITC-dextran permeability experiment. Food was removed 4 hours before oral gavage and withheld for the duration of the experiment. Water was removed immediately after FITC-dextran administration to control for variability in absorption and urinary excretion, then returned following blood collection.

At the time of gavage, mice received fluorescein isothiocyanate (FITC)-conjugated dextran (4 kDa; 100 mg/mL in sterile H_2O) at a dose of 0.6 mg/g body weight by oral gavage. Based on prior evidence indicating that FITC-dextran reaches the distal intestine within 4 hours of administration[102], blood was collected via retro-orbital bleed 4 hours post-gavage to obtain serum for quantification of circulating FITC-dextran as an index of intestinal paracellular permeability. At the time of tissue harvest, intestines were rapidly excised and placed in chilled 1× PBS. The small and large intestines were separated, and the luminal contents were gently flushed with cold PBS. Tissues were opened longitudinally and rinsed thoroughly. Mucosal scrapings were collected with glass slides, immediately placed on dry ice, snap-frozen in liquid nitrogen, and stored at −80 °C until subsequent molecular analyses.

RNA Isolation and Gene Expression Analyses

All work surfaces and materials used for RNA isolation were decontaminated with 70% ethanol and treated with an RNase inhibitor (RNase Out, G-Biosciences, St. Louis, MO, USA) to prevent RNA degradation. Total RNA was isolated from small intestinal (jejunal) and large intestinal mucosal scrapings using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were assessed prior to downstream applications. First-strand complementary DNA (cDNA) was synthesized from normalized total RNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry (Bio-Rad, Irvine, CA, USA) with PrimePCR Assays targeting CB1R (CNR1), CB2R (CNR2), OCLN (occludin), Tight junction protein 1 (TJP1/ZO-1), TNF

(TNFα), and IL1B (IL-1β). Reactions were run in duplicate, and relative gene expression was calculated using the comparative Ct method (2ΔΔCt). Housekeeping genes served as internal controls and were validated by confirming that expression was not affected across experimental conditions. Hprt was used as a housekeeping gene for the jejunum and large intestinal epithelium.

Plasma Lipopolysaccharide (LPS) Quantification

Circulating lipopolysaccharide levels were measured using a commercially available Mouse LPS ELISA Kit (CUSABIO, Cat# CSB-E13066m, Wuhan, China) according to the manufacturer's instructions. Whole blood was collected in EDTA-coated tubes and centrifuged at 1,000 × g for 15 minutes at 4°C to isolate plasma. Plasma samples were diluted 1:10 in the kit-provided sample diluent before analysis. Standards and samples were added to pre-coated microplate wells, and absorbance was measured with a microplate reader at the recommended wavelength. LPS concentrations were calculated from a standard curve generated with known concentrations of mouse LPS and expressed according to the manufacturer's specifications.

Multiplex Cytokine Analysis

Plasma cytokine concentrations were measured using the LEGENDplex™ Mouse Inflammation Panel (BioLegend, San Diego, CA, USA), a bead-based multiplex immunoassay designed for simultaneous quantification of 13 mouse inflammatory cytokines. This panel targets cytokines primarily produced by innate immune cells and mediators involved in innate–adaptive immune crosstalk. This analysis was performed exclusively on male plasma samples. Whole blood was collected into EDTA-coated tubes

and centrifuged at 1,000 x g for 15 minutes at 4°C to isolate plasma. Plasma samples were diluted 1:10 prior to analysis. The assay was performed according to the manufacturer's instructions. Briefly, fluorescence-encoded capture beads were incubated with standards and diluted plasma samples, followed by addition of biotinylated detection antibodies and streptavidin-phycoerythrin (PE). After washing, samples were acquired on a NovoCyte flow cytometer (Agilent Technologies, Santa Clara, CA, USA). Cytokine concentrations were calculated using the LEGENDplex™ data analysis software based on standard curves generated for each analyte.

Endocannabinoid Lipid Extraction from Intestinal Mucosa and Plasma

As previously described [42, 57, 103, 104], frozen jejunal small intestinal mucosal scrapings and large intestinal mucosal scrapings were weighed and homogenized in 1.0 mL of chilled methanol containing deuterated internal standards: [$^{2}H_{5}$]-2-arachidonoylglycerol (2-AG; 500 pmol), [$^{2}H_{4}$]-anandamide (AEA; 1 pmol), and [$^{2}H_{4}$]-oleoylethanolamide (OEA; 10 pmol). Lipids were extracted by adding 2.0 mL of chloroform, followed by phase separation with 1.0 mL of ultra-purified water (0.2 μm filtered). Samples were centrifuged at 1500 x g for 15 minutes at 4°C. The lower organic phase was collected and evaporated under a stream of nitrogen gas (99.998% purity). A second chloroform extraction (1.0 mL) was performed to maximize lipid recovery, followed by centrifugation under the same conditions and collection of the organic phase. Combined organic extracts were reconstituted in 2.0 mL chloroform and purified using open-bed silica gel column chromatography. Columns were eluted with a 9:1 chloroform: methanol solution to isolate monoacylglycerols (MAGs) and fatty acid ethanolamides (FAEs). Eluates were dried

under nitrogen and reconstituted in 200 µL methanol: chloroform (1:1, v/v) prior to quantification by UPLC–MS/MS. For plasma analyses, 100 µL of plasma was used per sample. Extraction procedures were identical to those described above, except that 900 µL of 0.9% NaCl was used in place of 1.0 mL ultra-purified water to facilitate phase separation.

UPLC-MS/MS Quantification of 2-AG and AEA

Endocannabinoid analyses were performed using an Acquity I-Class UPLC system coupled directly to a Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with electrospray ionization (ESI). Chromatographic separation was achieved using an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm; Waters) with an inline BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 µm; Waters). Analytes were eluted using a gradient of water and methanol (each containing 0.25% acetic acid and 5 mM ammonium acetate) at a flow rate of 0.4 mL/min. The gradient was as follows: 80% methanol (0.0–0.5 min), 80–100% methanol (0.5–2.5 min), 100% methanol (2.5–3.0 min), 100–80% methanol (3.0–3.1 min), and 80% methanol (3.1–4.5 min). Samples were maintained at 10°C in the autosampler, and the column temperature was held at 40°C. Argon (99.998%) was used as the collision gas. Mass spectrometric detection was performed in positive ion mode with a capillary voltage of 0.1 kV. Quantification of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) was achieved using multiple reaction monitoring (MRM) of the following transitions: AEA (m/z 348.3 → 62.0), [$^{2}H_{4}$]-AEA (m/z 352.3 → 66.1), 2-AG (m/z 379.3 → 287.3), and [$^{2}H_{5}$]-2-AG (m/z 384.3 → 93.4). Quantitation was performed using a stable isotope dilution

method, monitoring protonated molecular ions ($[M + H]^+$). Because acyl migration can occur during silica-gel purification of monoacylglycerols, total 2-AG levels are reported as the combined signal of 1-AG and 2-AG isomers [105].

Immunohistochemistry

Mid-to-distal large intestinal tissue was collected from intCB1R-/- and intCB1R+/+ control mice 7 days following completion of the tamoxifen induction schedule. Tissue was flushed with ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) and fixed for 4 hours at 4°C. Following fixation, tissues were cryoprotected, embedded in optimal cutting temperature (OCT) compound (Fisher Healthcare, Chino, CA, USA), and rapidly frozen on dry ice. Transverse sections (20 μm thickness) were cut using a cryostat (Leica, Wetzlar, Germany) and mounted onto charged glass slides. Sections were permeabilized with 0.5% Tween-20 in PBS and subsequently blocked in casein-based blocking solution containing 0.1% Tween-20 (Thermo Fisher Scientific, Waltham, MA, USA). Primary antibodies against CB1 receptor (rabbit anti-CB1; generously provided by Dr. Ken Mackie, Indiana University, Bloomington, IN, USA) and zonula occludens-1 (ZO-1 (1A12); mouse; Thermo Fisher Scientific, Waltham, MA, USA) were diluted 1:500 in blocking buffer. Sections were incubated with primary antibodies for 1 hour at room temperature. Following three washes with 0.1% Tween-20 in PBS, sections were incubated for 1 hour at room temperature with species-appropriate secondary antibodies conjugated to Alexa Fluor dyes (e.g., goat anti-rabbit Alexa Fluor 647 for CB1). After additional washes, coverslips were mounted using ProLong Gold Antifade reagent with DAPI (Thermo Fisher Scientific) for

nuclear counterstaining. Fluorescent images were acquired at room temperature using an Axio Observer Z1 inverted microscope (Zeiss, Oberkochen, Germany).

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Data were analyzed using unpaired two-tailed Student's t-tests, two-way ANOVA, or three-way ANOVA where appropriate based on experimental design (e.g., sex x genotype x treatment). When significant main effects or interactions were detected, Holm–Šidák multiple comparisons post hoc tests were applied. Data are presented as mean ± SEM, and statistical significance was defined as $p < 0.05$.

Results

1.1 Sex-dependent metabolic responses to Western diet in mice lacking intestinal CB1 receptors

To determine the impact of intestinal CB1 receptor deletion and cannabis exposure on metabolic responses during diet-induced obesity, male and female intCB1R+/+ and intCB1R-/- mice were fed a Western diet (WD) for 60 days, followed by 14 days of cannabinoid treatment (Figure 1A). In male intCB1R+/+ mice, WD exposure resulted in a significant increase in body weight over time compared to standard diet (SD)-fed controls (Figure 1B), demonstrating robust diet-induced obesity. Similarly, male intCB1R-/- mice exhibited significant weight gain in response to WD (Figure 1D), although the magnitude of weight gain was reduced compared to intCB1R+/+ mice.

During the 14-day treatment period, cannabinoid exposure significantly altered body weight in male intCB1R+/+ WD-fed mice (Figure 1C). Both Δ^9-THC and cannabis

extract reduced body weight gain, with cannabis extract producing a greater decrease relative to THC alone. In contrast, cannabinoid treatment had no effect on body weight in male intCB1R-/- WD-fed mice (Figure 1E), indicating that intestinal CB1 receptors contribute to cannabinoid-mediated metabolic effects in males.

In female mice, WD feeding increased body weight in intCB1R+/+ animals compared to SD controls (Figure 1F). However, this effect was absent in female intCB1R-/- mice, which did not show significant diet-induced weight gain (Figure 1H), indicating a sex-dependent role for intestinal CB1 receptors in metabolic regulation. Unlike males, cannabinoid treatment did not significantly alter body weight in female mice, regardless of genotype (Figure 1G, I). Together, these findings demonstrate that intestinal CB1 receptors contribute to diet-induced obesity and to cannabinoid-mediated metabolic regulation in a sex-dependent manner.

1.2 Intestinal CB1 receptors protect against Western diet–induced gut barrier dysfunction in a sex-dependent manner

To determine the role of intestinal CB1 receptors in regulating gut barrier function during diet-induced obesity, intestinal permeability was assessed using 4-kDa FITC-dextran (FITC-dextran) at baseline (week 0), after 8 weeks of dietary intervention, and following 14 days of cannabinoid treatment (week 10) (Figure 2). At baseline, no differences in intestinal permeability were observed between genotypes or diets in either sex (Figure 2A, E), indicating comparable barrier function prior to dietary intervention.

After 8 weeks of diet exposure, WD-fed male intCB1R+/+ mice exhibited increased intestinal permeability compared to SD controls. This effect was significantly exacerbated

in WD-fed male intCB1R-/- mice, which displayed increased levels of FITC-dextran levels across all groups, suggesting that intestinal CB1 receptors protect against diet-induced barrier dysfunction in males (Figure 2B). In females, WD feeding increased intestinal permeability in intCB1R+/+ mice; however, this effect was absent in intCB1R-/- mice (Figure 2F), demonstrating a sex-dependent role for intestinal CB1 receptors in regulating barrier integrity. Following cannabinoid treatment (week 10), Vehicle-treated WD-fed male intCB1R+/+ mice maintained elevated intestinal permeability compared to SD controls (Figure 2C). Although not statistically significant, cannabinoid treatment largely reversed this effect in these mice. In contrast, WD-fed male intCB1R-/- mice exhibited markedly elevated permeability under vehicle conditions, which was significantly reduced by both Δ^9-THC and whole cannabis extract, indicating that cannabinoids can restore barrier function independent of intestinal CB1 receptors in males. Consistent with this, analysis of the change in permeability from week 8 to week 10 showed that cannabinoid treatment reduced FITC-dextran levels in WD-fed male mice, particularly in intCB1R-/- animals (Figure 2D).

In females, WD-fed intCB1R+/+ mice showed increased intestinal permeability under vehicle treatment at week 10 (Figure 2G), and this increase was significantly reduced by cannabinoid treatment. In contrast, female intCB1R-/- mice showed no significant differences across diet or treatment groups. Analysis of the change in permeability from week 8 to week 10 further confirmed that cannabinoids significantly reduced permeability in WD-fed female intCB1R+/+ mice, whereas no effect was observed in intCB1R-/- mice (Figure 2H).

Together, these findings demonstrate that intestinal CB1 receptors play a protective role against diet-induced gut barrier dysfunction in a sex-dependent manner and that cannabinoids can restore barrier integrity through mechanisms that do not necessarily require signaling through intestinal CB1 receptors.

1.3 Loss of intestinal CB1 receptors exacerbates endotoxemia in males, and cannabis mitigates LPS translocation in a sex-dependent manner

To determine whether changes in intestinal permeability were associated with systemic endotoxemia, circulating levels of LPS were measured in plasma (Figure 3).

In male mice, loss of intestinal CB1 receptors significantly increased circulating LPS levels (Figure 3A), indicating exacerbated endotoxemia in intCB1R-/- mice compared to intCB1R+/+ controls. While WD-fed intCB1R+/+ mice had elevated LPS levels relative to SD controls, and cannabinoid treatment largely reduced LPS concentrations, though this effect did not reach statistical significance. These findings suggest that intestinal CB1 receptors play a protective role against endotoxemia in males and that cannabinoids may partially mitigate LPS translocation.

In contrast, no significant differences in circulating LPS levels were observed in female mice across genotypes, diets, or treatment conditions (Figure 3B), indicating that endotoxemia was not altered in females under these conditions.

Together, these results demonstrate that intestinal CB1 receptors protect against diet-induced endotoxemia in a sex-dependent manner and that cannabinoids may attenuate LPS translocation in males.

1.4 Cannabinoids restore jejunal tight junction gene expression in male diet-induced obesity

To determine whether cannabinoid-mediated improvements in gut barrier function were associated with changes in tight junction regulation, gene expression of occludin and ZO-1 was assessed in the jejunum and the large intestine (Figure 4).

In the jejunum of male mice, WD feeding significantly reduced occludin expression in both intCB1R+/+ and intCB1R-/- mice relative to SD-fed controls (Figure 4A). Cannabinoid treatment restored occludin expression in WD-fed intCB1R+/+ mice, with both Δ^9-THC and whole cannabis extract reversing this downregulation. A similar effect was observed in intCB1R-/- mice; however, this effect did not reach statistical significance, suggesting that cannabinoid-mediated restoration of OCLN expression is more robust in the presence of intestinal CB1 receptors.

In contrast, ZO-1 expression in the jejunum of male mice was primarily influenced by genotype (Figure 4B). SD-fed intCB1R-/- mice showed increased ZO-1 expression relative to controls, and cannabinoid treatment further increased ZO-1 expression in WD-fed intCB1R-/- mice. No significant changes were observed in intCB1R+/+ mice across diet or treatment conditions.

In female mice, jejunal occludin expression was reduced in WD-fed intCB1R+/+ vehicle-treated mice relative to SD controls, and this effect was reversed by cannabinoid treatment (Figure 4C). However, no significant changes were observed in intCB1R-/- mice. Jejunal ZO-1 expression remained unchanged across all conditions in females (Figure 4D).

In the large intestine, distinct genotype-dependent effects were observed in males. Occludin expression was significantly reduced across all intCB1R-/- groups, regardless of diet or treatment, whereas expression remained stable in intCB1R+/+ mice (Figure 4E). ZO-1 expression in the large intestine of male mice remained largely unchanged across all groups (Figure 4F). In females, neither occludin nor ZO-1 expression in the large intestine was significantly altered by diet, genotype, or cannabinoid treatment (Figure 4G, H).

Together, these findings demonstrate that cannabinoids restore tight junction gene expression in a region- and sex-dependent manner, with the most pronounced effects observed in the jejunum of male mice under diet-induced obesity.

1.5 Cannabinoids restore inflammatory homeostasis in a sex-dependent and region-specific manner during diet-induced obesity

To determine whether cannabinoid-mediated improvements in gut barrier function were associated with changes in inflammatory signaling, expression of the pro-inflammatory cytokines TNFα and IL-1β was assessed in the jejunum and large intestine (Figure 5).

In the jejunum of male mice, WD feeding significantly increased TNFα expression in intCB1R+/+ vehicle-treated mice relative to SD controls (Figure 5A). Both Δ^9-THC and whole cannabis extract significantly reversed this effect, restoring TNFα expression toward baseline levels. In contrast, intCB1R-/- mice exhibited reduced TNFα expression across both SD- and WD-fed conditions, despite increased intestinal permeability in these animals, suggesting a potential dissociation between barrier dysfunction and local inflammatory signaling in the absence of intestinal CB1 receptors.

A similar pattern was observed in IL-1β expression in the jejunum of male mice (Figure 5B). WD feeding increased IL-1β expression in intCB1R+/+ vehicle-treated mice, and both cannabinoid treatments significantly reversed this effect. In contrast, intCB1R-/- mice exhibited reduced IL-1β expression regardless of diet, further supporting a genotype-dependent alteration in inflammatory signaling.

In female mice, jejunal TNFα expression was reduced across multiple conditions relative to SD controls (Figure 5C), indicating a distinct inflammatory profile compared to males. Jejunal IL-1β expression remained unchanged across all groups in females (Figure 5D).

In the large intestine of male mice, pro-inflammatory cytokine expression differed from that in the jejunum. TNFα expression was reduced in WD-fed mice regardless of genotype or treatment, and was also decreased in SD-fed intCB1R-/- mice (Figure 5E). In contrast, IL-1β expression remained unchanged across all groups in the large intestine of male mice (Figure 5F).

In female mice, TNFα expression in the large intestine was largely unchanged, except in WD-fed intCB1R-/- mice treated with cannabis extract, which showed a reduction (Figure 5G). For IL-1β, WD-fed intCB1R+/+ vehicle-treated mice exhibited increased expression, which was significantly reversed by Δ^9-THC (Figure 5H). No significant changes were observed in intCB1R-/- mice.

Given the observed increase in intestinal permeability and endotoxin translocation, we next asked whether these changes were associated with systemic immune activation. To address this, circulating cytokines were quantified in plasma using a multiplex cytokine

assay. In male mice, WD feeding increased circulating IL-1α levels in vehicle-treated groups, and this effect was largely reversed by cannabinoid treatment in both intCB1R+/+ and intCB1R−/− mice. However, no robust differences were observed in other circulating cytokines across diet, genotype, or treatment conditions (Supplemental Figure 1.2).

Together, these findings demonstrate that cannabinoids restore inflammatory homeostasis in a sex- and region-specific manner, with pronounced anti-inflammatory effects observed in the jejunum of male mice during diet-induced obesity.

1.6 Diet-induced obesity drives region-specific and sex-dependent dysregulation of the endocannabinoid system

To determine whether alterations in gut barrier function and inflammation were associated with dysregulation of the endocannabinoid system, levels of 2-AG and AEA were quantified in plasma, jejunum epithelium, and large intestinal epithelium (Figure 6).

In male mice, WD feeding significantly increased plasma 2-AG levels in both intCB1R+/+ and intCB1R-/- mice relative to SD controls (Figure 6A). Δ^9-THC treatment reduced plasma 2-AG levels in WD-fed intCB1R+/+ mice, whereas no effect was observed in intCB1R-/- mice. In the jejunum, WD feeding increased 2-AG levels in intCB1R+/+ vehicle-treated mice, and this increase was reversed by Δ^9-THC (Figure 6B). In the large intestine, 2-AG levels were reduced in WD-fed intCB1R+/+ vehicle-treated mice relative to SD controls, an effect not observed in cannabinoid-treated groups (Figure 6C). No significant changes in 2-AG levels were observed in intCB1R-/- mice in the large intestine.

In contrast, female mice showed no significant changes in 2-AG levels in plasma, jejunum, or large intestine, regardless of diet, genotype, or treatment (Figure 6D–F),

highlighting sex-specific dysregulation of 2-AG signaling in response to diet-induced obesity.

Male mice fed WD showed increased plasma AEA levels in intCB1R+/+ vehicle-treated mice, and these increases were reversed by both Δ^9-THC and whole cannabis extract (Figure 6G). No significant changes in AEA levels were observed in the jejunum or large intestine of male mice (Figure 6H, I).

In female mice, AEA levels remained largely unchanged in plasma and the jejunum (Figure 6J, K). In the large intestine, AEA levels were significantly higher in intCB1R-/- mice than in intCB1R+/+ controls (Figure 6L).

Together, these findings demonstrate that diet-induced obesity drives region-specific and sex-dependent dysregulation of the eCB system, with pronounced alterations in 2-AG and AEA signaling observed in male mice and distinct genotype-dependent effects in females.

1.7 Cannabinoid receptor expression in the intestine exhibits region-specific and sexually dimorphic changes during diet-induced obesity

To determine whether alterations in endocannabinoid tone were associated with changes in receptor expression, gene expression of the cannabinoid receptors CNR1 and CNR2 was assessed in the jejunum epithelium and the large intestinal epithelium (Figure 7).

In the jejunum, CNR1 expression was significantly reduced in all intCB1R-/- mice, across both males and females, confirming the efficiency of intestinal epithelial-specific CB1 receptor deletion (Figure 7A, C). In contrast, CNR1 expression remained stable across

all intCB1R+/+ groups, regardless of diet; however, cannabis extract treatment reduced CNR1 expression in female intCB1R+/+ mice.

Jejunal CNR2 expression exhibited distinct genotype- and treatment-dependent regulation in male mice (Figure 7B). WD-fed intCB1R+/+ vehicle-treated mice displayed increased CNR2 expression relative to SD controls, and both Δ^9-THC and whole cannabis extract significantly reversed this effect. Additionally, intCB1R-/- mice exhibited reduced CNR2 expression compared to controls. In contrast, no significant changes in jejunal CNR2 expression were observed in female mice (Figure 7D).

In the large intestine, CNR1 expression was significantly reduced in all intCB1R-/- mice in both sexes, further confirming knockout specificity (Figure 7E, G). Notably, male WD-fed intCB1R+/+ mice also exhibited reduced CNR1 expression in the colon, an effect not observed in females, indicating a sex-specific downregulation of CB1 signaling in response to diet-induced obesity.

CNR2 expression in the large intestine remained unchanged across all groups in both male and female mice (Figure 7F, H).

Together, these findings demonstrate that cannabinoid receptor expression is region-specific and sexually dimorphic, with diet-induced obesity selectively altering CB1 and CB2 signaling in the male intestine while leaving female receptor expression largely unchanged.

Discussion

Diet-induced obesity is increasingly recognized as a disorder that extends beyond systemic metabolism to include profound alterations in intestinal physiology. In this study,

we demonstrate that intestinal CB1R signaling plays a critical, sex-dependent role in regulating gut barrier function during diet-induced obesity. Cannabinoid exposure restores barrier integrity through both CB1-dependent and CB1-independent mechanisms. These findings provide new insight into how endocannabinoid signaling integrates metabolic and intestinal physiology under obesogenic conditions.

A central finding of this study is that intestinal CB1R signaling exerts sex-dependent effects on both metabolic and barrier phenotypes, revealing fundamentally distinct regulatory mechanisms in males and females. In male mice, loss of intestinal CB1R attenuated weight gain during Western diet feeding (Figure 1D), consistent with prior work showing that peripheral CB1R signaling promotes hyperphagia and energy storage [64]. Additionally, these results also align with previous studies showing that deletion of intestinal CB1R is associated with exacerbated levels of FITC-Dextran levels, elevated expression of key inflammatory genes, and reduced expression of gut barrier–associated proteins, suggesting that CB1 receptors in the intestinal epithelium play a protective role in maintaining barrier function during diet-induced obesity [42]. However, this metabolic improvement occurred alongside exacerbated intestinal permeability (Figure 2B) and elevated circulating LPS (Figure 3A), indicating that CB1R signaling is protective for barrier integrity despite its obesogenic effects. These findings highlight a functional tradeoff in which intestinal CB1R promotes metabolic dysfunction while preserving epithelial barrier function in males.

In contrast, female mice lacking intestinal CB1R were resistant to WD-induced weight gain (Figure 1H) without increased permeability (Figure 2F-H) or endotoxemia

(Figure 3B), indicating that CB1R is not necessarily required for maintaining barrier integrity in females. Moreover, cannabinoid treatment had minimal effects on both metabolic and barrier outcomes in females compared to males, with the exception of improved permeability (Figure 2G-H). These findings suggest that females engage CB1R-independent mechanisms that maintain intestinal homeostasis, potentially through sex hormone-mediated regulation of immune responses, epithelial function, or endocannabinoid signaling [22, 23, 80, 92, 106-109]. Together, these results establish sex as a primary determinant of how intestinal CB1R signaling coordinates metabolic and barrier function.

Importantly, cannabinoid treatment restored intestinal barrier function across multiple conditions, including in both male intCB1+/+ and intCB1R-/- mice (Figure 2C-D), indicating that these effects are not mediated exclusively by epithelial CB1R signaling. Both Δ^9-THC and whole cannabis extract reduced intestinal permeability and attenuated LPS translocation (Figure 2C-D; Figure 3A), particularly in WD-fed males. These findings suggest the involvement of CB1-independent mechanisms, potentially including signaling through CB2 receptors, TRP channels, or PPARs, as well as indirect effects on immune cells or enteric neurons [48]. Additionally, cannabinoids may influence gut barrier function by modulating the gut microbiota or microbial metabolites, which are known regulators of epithelial integrity [29, 110].

Notably, whole cannabis extract often produced equal or greater effects than Δ^9-THC alone, particularly in reducing body weight (Figure 1C) and improving barrier function, especial ly in male intCB1-/- mice. These findings align with our recent co–first

author study [111], which demonstrated that chronic (30-day) exposure to Δ9-THC and whole cannabis extract reduced body weight, decreased fat mass, and improved glucose tolerance in WD-fed male mice. The enhanced efficacy of the whole extract suggests a potential "entourage effect," in which non-THC phytocannabinoids such as CBD contribute to the observed physiological outcomes. CBD has been shown to exert anti-inflammatory effects, reduce pro-inflammatory cytokine production, and improve epithelial barrier function in vitro and in vivo [112-115]. Importantly, CBD has minimal direct activity at CB1 receptors and instead exerts its effects through alternative signaling pathways, including modulation of inflammatory signaling and non-cannabinoid receptor targets. This supports the hypothesis that cannabinoid-mediated improvements in barrier function involve mechanisms beyond classical CB1 activation. Future studies extending chronic cannabinoid exposure paradigms to intestinal permeability assays will be important for determining whether long-term improvements in metabolic health are directly linked to sustained restoration of barrier integrity.

Our data also reveal a dissociation between intestinal barrier dysfunction and local inflammatory gene expression in male intCB1R-/- mice. Despite increased permeability (Figure 2B–D) and elevated circulating LPS (Figure 3A), these mice paradoxically exhibited reduced jejunal and colonic expression of TNFα and IL-1β (Figure 5A–B, E–F). This finding suggests that barrier dysfunction in this model is not solely driven by local inflammatory signaling but may instead reflect structural alterations in tight junction organization. Tight junction proteins such as occludin and ZO-1 are key regulators of the leak pathway, which mediates the passage of macromolecules across the epithelium [18].

Importantly, the cannabinoid-mediated restoration of occludin was highly context-dependent. In the jejunum of WD-fed male intCB1R+/+ mice, both Δ^9-THC and cannabis extract restored occludin expression (Figure 4A) and normalized pro-inflammatory cytokines TNFα and IL-1β (Figure 5A–B). However, these restorative effects were absent or blunted in intCB1R-/- mice, indicating that epithelial CB1R signaling is required for full transcriptional recovery of tight junction and inflammatory pathways. Together, these findings suggest that while cannabinoids can improve barrier function through CB1-independent mechanisms, optimal regulation of tight junction expression and inflammatory homeostasis in the jejunum may require intact CB1R signaling.

Region-specific effects further support this interpretation. Cannabinoid-mediated improvements in tight junction expression and inflammatory signaling were most pronounced in the jejunum (Figure 4A–D; Figure 5A–D), whereas the large intestine exhibited relatively modest or distinct responses (Figure 4E–H; Fig. 5E–H). The small intestine, particularly the jejunum, is a key site of nutrient sensing and endocannabinoid (eCB) signaling, and has been shown to play a central role in regulating feeding behavior and metabolic responses (DiPatrizio, 2021). Importantly, barrier organization differs substantially along the gastrointestinal tract. In the small intestine, where nutrient absorption is prioritized, barrier function relies heavily on epithelial defenses, including AMPs secreted by Paneth cells, which limit direct contact between luminal microbes and the epithelium. In contrast, the large intestine harbors a dense and diverse microbial population and therefore relies more on a robust physical separation system, characterized by a thick, stratified inner mucus layer composed of polymerized MUC2 that spatially

segregates bacteria from epithelial cells [21, 95, 96]. Additional mechanisms, such as epithelial-derived proteins that restrict bacterial motility, further reinforce this separation. These regional differences in barrier architecture and microbial exposure create distinct regulatory environments along the intestine. In this context, our findings reinforce that intestinal barrier regulation is region-specific, with the jejunum serving as a key site where metabolic signaling, including the eCB system, influences barrier function.

In parallel with these functional changes, we observed pronounced dysregulation of the eCB system in a sex- and region-specific manner (Figure 6). WD feeding increased 2-arachidonoylglycerol (2-AG) levels in plasma from male mice (Figure 6A) and anandamide (AEA) levels in plasma and jejunal tissue from male intCB1+/+ mice (Figure B, G), as well as reduced 2-AG in the large intestine (Figure 6C). By contrast, female mice exhibited minimal changes in eCB tone (Figure 6D–F, J–L). These findings are consistent with previous reports demonstrating that obesity is associated with elevated endocannabinoid levels and increased CB1R signaling activity [45, 46]. Notably, elevated 2-AG has been implicated in both pro- and anti-inflammatory processes, depending on context. For example, increasing endogenous 2-AG levels has been shown to protect against colitis and reduce systemic inflammation [59], suggesting that eCB signaling may play a compensatory role in maintaining intestinal homeostasis. Thus, the observed increases in eCB tone in male mice may reflect an adaptive response to WD-induced stress that becomes dysregulated during chronic metabolic disease.

Consistent with these changes, cannabinoid receptor expression exhibited region-specific and sexually dimorphic regulation (Figure 7). In male intCB1+/+ mice, WD

feeding reduced colonic CNR1 expression (Figure 7E), while jejunal CNR2 expression was increased and normalized by cannabinoid treatment (Fig. 7B). The observed upregulation of CNR2 in the jejunum of WD-fed male intCB1+/+ mice likely reflects a compensatory response to increased intestinal inflammation and immune activation. Cannabis treatment reversed this effect, suggesting a reduction in inflammatory tone and a normalization of endocannabinoid signaling within the intestine. In contrast, female mice showed minimal changes in receptor expression across conditions (Fig. 7C–D, G–H). These findings further support the concept that eCB signaling is differentially regulated across intestinal regions and between sexes, which may contribute to the observed variability in barrier and metabolic responses.

An additional layer of regulation may involve intestinal alkaline phosphatase (IAP), a key enzyme that detoxifies LPS and maintains barrier integrity. IAP activity is reduced in obesity and contributes to metabolic endotoxemia and inflammation [75, 76]. Given that both IAP and cannabinoid signaling regulate LPS translocation and barrier function, these systems may interact to coordinate intestinal homeostasis. Although not directly assessed in the present study, the observation that cannabinoids reduce circulating LPS (Figure 3A) raises the possibility that CB1 signaling may influence IAP expression or activity, representing an additional mechanism by which cannabinoid signaling protects against endotoxemia. This is further supported by human data showing that obese individuals have increased circulating AEA together with reduced duodenal expression of IAP and ZO-1, and that plasma AEA is negatively associated with both IAP and ZO-1 expression,

suggesting a link between altered endocannabinoid signaling, impaired barrier regulation, and metabolic inflammation in obesity[78].

Collectively, our findings establish sex as a critical biological variable in regulating intestinal barrier function and cannabinoid responsiveness during metabolic disease. Male mice exhibited pronounced alterations in body weight, intestinal permeability, endotoxemia, inflammatory signaling, and eCB tone (Figures 1–7), whereas female mice were largely protected from these disruptions. These differences likely reflect coordinated effects of sex hormones, immune regulation, and endocannabinoid signaling pathways [80, 92]. Emerging evidence further suggests that these sex-dependent effects may arise, at least in part, from intrinsic signaling differences within the intestinal epithelium itself. For example, epithelial-specific deletion of ERα protects female mice from intestinal injury while exacerbating inflammation and epithelial dysfunction in males, demonstrating that identical epithelial pathways can produce opposing outcomes depending on sex[24]. In that study, male ERα-deficient colonoids also showed reduced expression of multiple epithelial functional genes, including tight junction-associated transcripts, whereas female colonoids displayed a more protective gene signature. Importantly, the blunted response to both diet-induced stress and cannabinoid intervention in females suggests fundamentally distinct regulatory mechanisms governing intestinal homeostasis. Given that many prior studies have focused predominantly on male models, these findings highlight the importance of incorporating sex as a biological variable when investigating cannabinoid signaling and gut barrier function.

Limitations and Future Directions

To further define the mechanisms by which cannabinoid signaling regulates intestinal barrier function, several key areas warrant investigation. First, future studies will refine the assessment of intestinal permeability by measuring 4 kDa FITC-dextran at 2 hours post–oral gavage to specifically assess small intestinal permeability, complementing the 4-hour measurements used in the present study to provide a more comprehensive regional permeability profile. Second, the role of sex hormones in eCB-mediated barrier regulation will be examined through ovariectomy studies in female mice. These experiments will determine the contribution of sex hormones to endocannabinoid content, body weight regulation, tight junction protein expression, and localization. Third, the role of CB2 signaling in gut barrier integrity will be defined using intestinal CB2 knockout models. These studies will assess the impact of CB2 signaling on intestinal permeability, LPS translocation, tight junction regulation, and eCB activity. Fourth, future work will investigate the role of IAP in cannabinoid-mediated barrier regulation by examining whether CB1 signaling influences IAP expression and activity, and whether altered IAP function represents an additional mechanism by which CB1 protects against endotoxemia and barrier dysfunction. Finally, given the metabolic improvements observed in our recent study (Avalos, Olmos et al., 2026), future experiments will extend cannabinoid exposure to 30-day paradigms and assess intestinal permeability alongside metabolic outcomes to determine whether long-term improvements in body weight, adiposity, and glucose tolerance are associated with sustained restoration of gut barrier function. Together, these

studies will further define how cannabinoid signaling, sex, and diet converge to regulate intestinal barrier integrity and immune homeostasis in metabolic disease.

Figures and tables

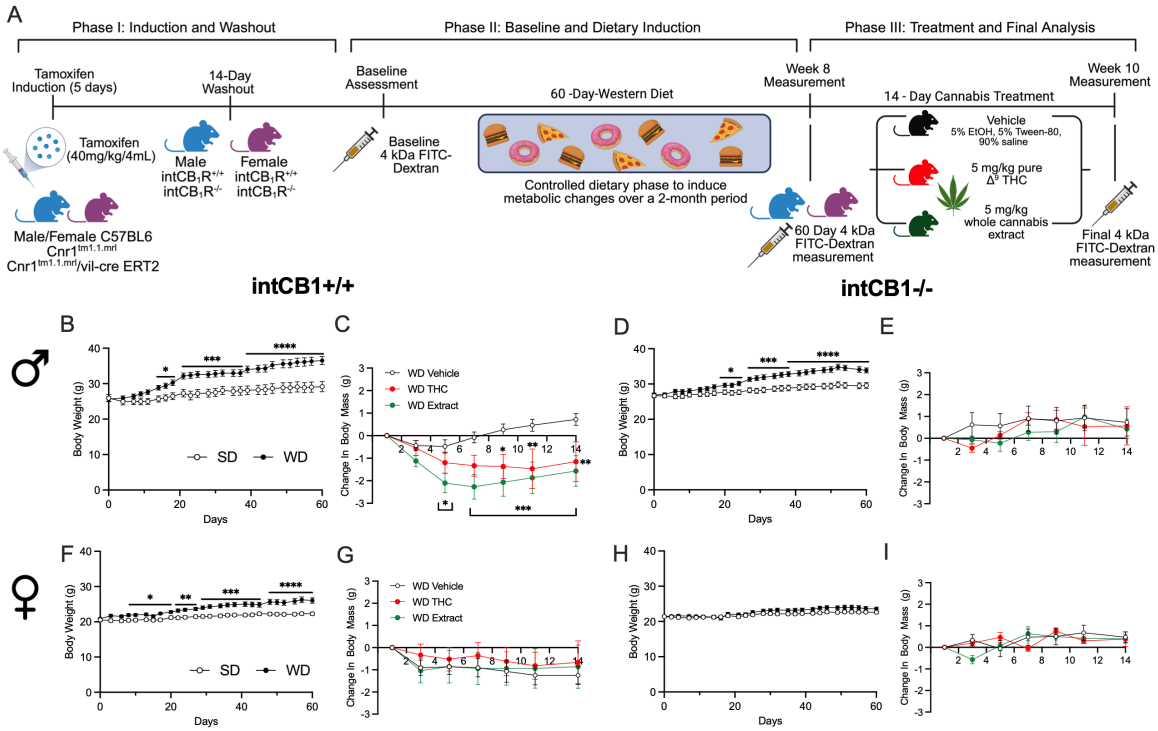

A
Phase I: Induction and Washout
Phase II: Baseline and Dietary Induction
Phase III: Treatment and Final Analysis
Tamoxifen Induction (5 days)
14-Day Washout
Baseline Assessment
60 -Day-Western Diet
Week 8 Measurement
14 - Day Cannabis Treatment
Week 10 Measurement
Tamoxifen (40mg/kg/4mL)
Male intCB1R+/+ intCB1R-/-
Female intCB1R+/+ intCB1R-/-
Baseline 4 kDa FITC-Dextran
Controlled dietary phase to induce metabolic changes over a 2-month period
Vehicle 5% EtOH, 5% Tween-80, 90% saline
5 mg/kg pure Δ9 THC
5 mg/kg whole cannabis extract
Male/Female C57BL6 Cnr1tm1.1.mrl Cnr1tm1.1.mrl/vil-cre ERT2
60 Day 4 kDa FITC-Dextran measurement
Final 4 kDa FITC-Dextran measurement
intCB1+/+
intCB1-/-
B
C
D
E
F
G
H
I
Body Weight (g)
Days
SD
WD
Change In Body Mass (g)
WD Vehicle
WD THC
WD Extract

Figure 1. 1: Sex-dependent metabolic responses to Western diet in mice lacking intestinal CB1 receptors

(A) Experimental design. Male and female intCB1R+/+ and intCB1R-/- mice underwent tamoxifen-induced recombination followed by a 14-day washout period. Intestinal permeability was assessed at baseline (week 0) prior to 60 days of dietary intervention (standard diet [SD] or Western diet [WD]). After 8 weeks on diet, intestinal permeability was reassessed, and mice were weight-matched and treated for 14 days with vehicle, Δ9-tetrahydrocannabinol (THC; 5 mg/kg), or whole cannabis extract (5 mg/kg) matched for THC content. Final intestinal permeability measurements were obtained at week 10, followed by tissue collection for downstream analyses. **(B)** Body weight over time in male intCB1R+/+ mice fed SD or WD. Two-way ANOVA revealed significant effects of time ($F(25,729) = 10.72$, $p < 0.0001$), diet ($F(1,729) = 275.0$, $p < 0.0001$), and time x diet interaction ($F(25,729) = 1.979$, $p = 0.0031$). n = 13-18 per group. **(C)** Change in body weight during 14-day treatment in male intCB1R+/+ WD-fed mice. Two-way ANOVA showed significant effects of time ($F(6,98) = 2.315$, $p = 0.0392$) and drug ($F(2,98) = 18.68$, $p < 0.0001$), with no interaction ($F(12,98) = 1.053$, $p = 0.4079$). n = 5-6 per group. **(D)** Body weight over time in male intCB1R-/- mice fed SD or WD. Two-way ANOVA revealed significant effects of time ($F(25,1114) = 13.64$, $p < 0.0001$), diet ($F(1,1114) = 235.5$, $p < 0.0001$), and interaction ($F(25,1114) = 2.208$, $p = 0.0006$). n = 22-23 per group. **(E)** Change in body weight during 14-day treatment in male intCB1R-/- WD-fed mice. No significant effects of time ($F(6,133) = 0.8148$, $p = 0.5602$), drug ($F(2,133) = 0.8923$, $p = 0.4122$), or interaction ($F(12,133) = 0.1826$, $p = 0.9989$). n = 5-12 per group. **(F)** Body weight over time in female intCB1R+/+ mice fed SD or WD. Two-way ANOVA revealed significant effects of time ($F(25,1092) = 10.63$, $p < 0.0001$), diet ($F(1,1092) = 284.3$, $p < 0.0001$), and interaction ($F(25,1092) = 1.736$, $p = 0.0140$). n = 22 per group. **(G)** Change in body weight during 14-day treatment in female intCB1R+/+ WD-fed mice. No significant effects of time ($F(6,126) = 1.081$, $p = 0.3775$), drug ($F(2,126) = 1.125$, $p = 0.3280$), or interaction ($F(12,126) = 0.07320$, $p > 0.9999$). n = 5-11 per group. **(H)** Body weight over time in female intCB1R-/- mice fed SD or WD. Two-way ANOVA revealed a significant effect of time ($F(26,1052) = 8.650$, $p < 0.0001$) and diet ($F(1,1052) = 19.57$, $p < 0.0001$), with no interaction ($F(26,1052) = 0.6771$, $p = 0.8878$). n = 21 per group. **(I)** Change in body weight during 14-day treatment in female intCB1R-/- WD-fed mice. No significant effects of time ($F(6,126) = 1.743$, $p = 0.1162$), drug ($F(2,126) = 0.3816$, $p = 0.6836$), or interaction ($F(12,126) = 0.8023$, $p = 0.6475$). n = 5-10 per group. Data are presented as mean ± SEM. Statistical analyses were performed using two-way ANOVA followed by Holm–Šidák post hoc tests.

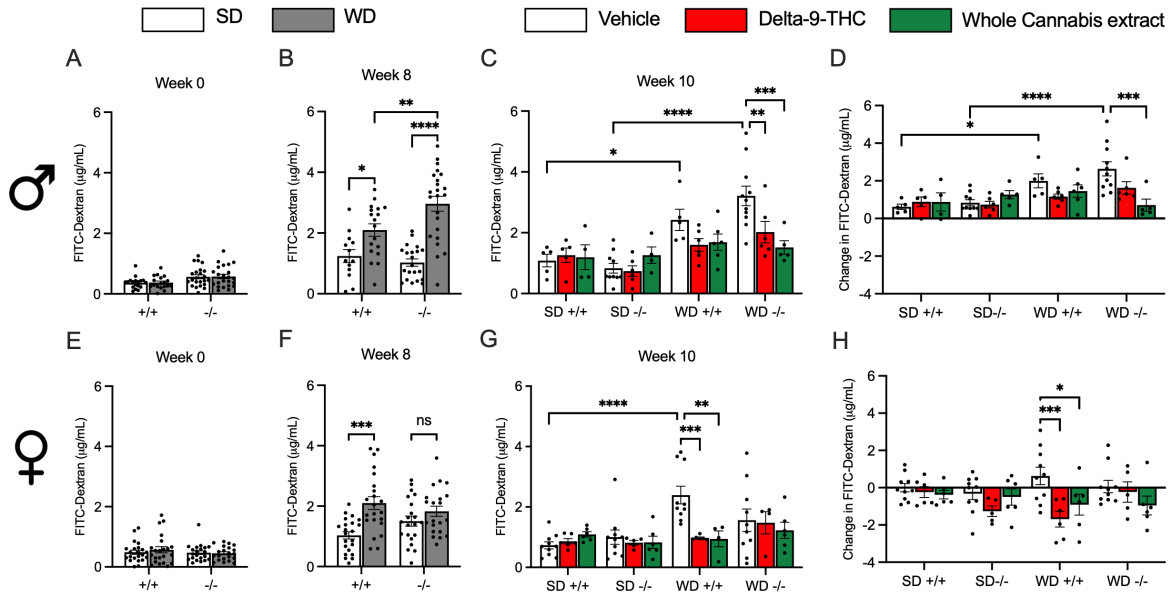

SD
WD
Vehicle
Delta-9-THC
Whole Cannabis extract
A
B
C
D
E
F
G
H
Week 0
Week 8
Week 10
FITC-Dextran (µg/mL)
Change in FITC-Dextran (µg/mL)
+/+
-/-
SD +/+
SD -/-
SD-/-
WD +/+
WD -/-
ns

Figure 1. 2: Intestinal CB1 receptors protect against Western diet–induced gut barrier dysfunction in a sex-dependent manner

(A) Baseline (week 0) intestinal permeability in male intCB1R+/+ and intCB1R-/- mice fed SD or WD. Two-way ANOVA revealed a significant effect of genotype ($F(1,78) = 9.914$, $p = 0.0023$), with no effect of diet ($F(1,78) = 0.01392$, $p = 0.9064$) and no interaction ($F(1,78) = 0.001466$, $p = 0.9696$). n = 18-23 per group. **(B)** Week 8 intestinal permeability in male mice. Two-way ANOVA revealed a significant effect of genotype ($F(1,71) = 43.93$, $p < 0.0001$), with no effect of diet ($F(1,71) = 2.406$, $p = 0.1253$), and a significant interaction ($F(1,71) = 6.595$, $p = 0.0123$). n = 13-23 per group. **(C)** Week 10 intestinal permeability in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,61) = 0.1044$, $p = 0.7477$), a significant effect of diet ($F(1,61) = 34.91$, $p < 0.0001$), and a significant effect of drug ($F(2,61) = 3.797$, $p = 0.0279$). A significant drug x diet interaction was observed ($F(2,61) = 6.896$, $p = 0.0020$). n = 5-11 per group. **(D)** Change in intestinal permeability (week 8 to week 10) in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,62) = 0.5372$, $p = 0.4663$), a significant effect of diet ($F(1,62) = 15.56$, $p = 0.0002$), and no effect of drug ($F(2,62) = 2.667$, $p = 0.0774$). A significant drug x diet interaction was observed ($F(2,62) = 6.529$, $p = 0.0027$). n = 5-11 per group. **(E)** Baseline (week 0) intestinal permeability in female mice. Two-way ANOVA revealed no effect of genotype ($F(1,82) = 0.7772$, $p = 0.3806$), no effect of diet ($F(1,82) = 0.1622$, $p = 0.6882$), and no interaction ($F(1,82) = 0.4973$, $p = 0.4827$). n = 21-22 per group. **(F)** Week 8 intestinal permeability in female mice. Two-way ANOVA revealed a significant effect of genotype ($F(1,82) = 16.91$, $p < 0.0001$), no effect of diet ($F(1,82) = 0.3505$, $p = 0.5555$), and a significant interaction ($F(1,82) = 4.814$, $p = 0.0311$). n = 21-22 per group. **(G)** Week 10 intestinal permeability in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,71) = 0.008969$, $p = 0.9248$), a significant effect of diet ($F(1,71) = 11.74$, $p = 0.0010$), and a significant effect of drug ($F(2,71) = 3.403$, $p = 0.0388$). Significant interactions were observed for drug x diet ($F(2,71) = 4.265$, $p = 0.0178$) and drug x diet x genotype ($F(2,71) = 3.753$, $p = 0.0282$). n = 5-10 per group. **(H)** Change in intestinal permeability (week 8 to week 10) in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,74) = 0.1940$, $p = 0.6609$), no effect of diet ($F(1,74) = 0.07957$, $p = 0.7787$), and a significant effect of drug ($F(2,74) = 7.195$, $p = 0.0014$). A significant drug x genotype interaction was observed ($F(2,74) = 3.137$, $p = 0.0492$). n = 5-11 per group. Data are presented as mean ± SEM. Statistical analyses were performed using two-way ANOVA followed by Holm-Sidak post hoc tests.

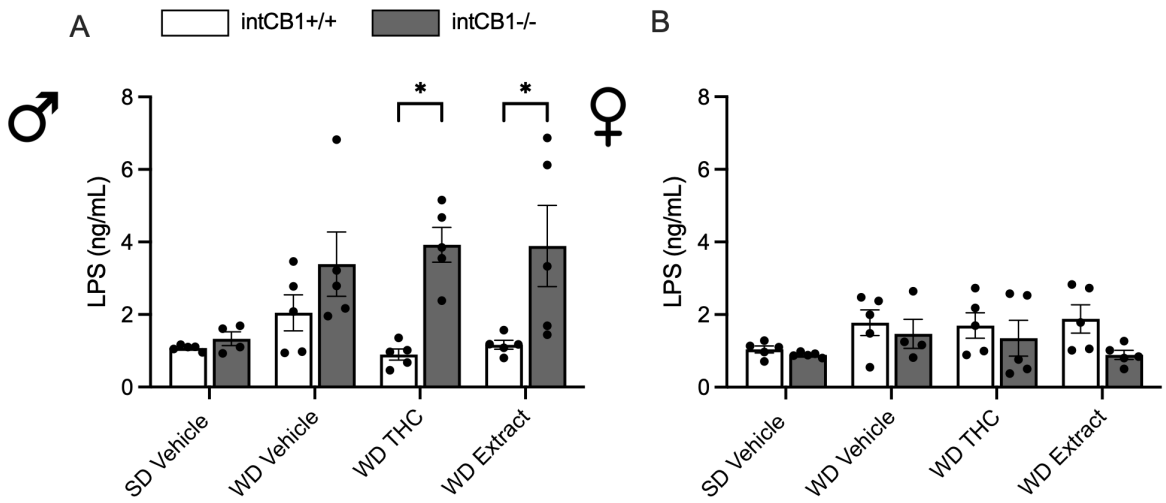


Figure 1. 3: Loss of intestinal CB1 receptors exacerbates endotoxemia in males, and cannabis mitigates LPS translocation in a sex-dependent manner

(A) Plasma LPS levels in male intCB1R+/+ and intCB1R-/- mice across treatment groups. Two-way ANOVA revealed a significant effect of genotype ($F(1,31) = 19.72$, $p = 0.0001$), with no effect of drug ($F(3,31) = 2.596$, $p = 0.0702$) and no interaction ($F(3,31) = 2.343$, $p = 0.0923$). n = 4-5 per group. **(B)** Plasma LPS levels in female intCB1R+/+ and intCB1R-/- mice across treatment groups. Two-way ANOVA revealed no effect of genotype ($F(1,31) = 3.973$, $p = 0.0551$), no effect of drug ($F(3,31) = 1.647$, $p = 0.1987$), and no interaction ($F(3,31) = 0.6974$, $p = 0.5608$). n = 4-5 per group. Data are presented as mean ± SEM. Statistical analyses were performed using two-way ANOVA followed by Holm-Sidak post hoc tests.

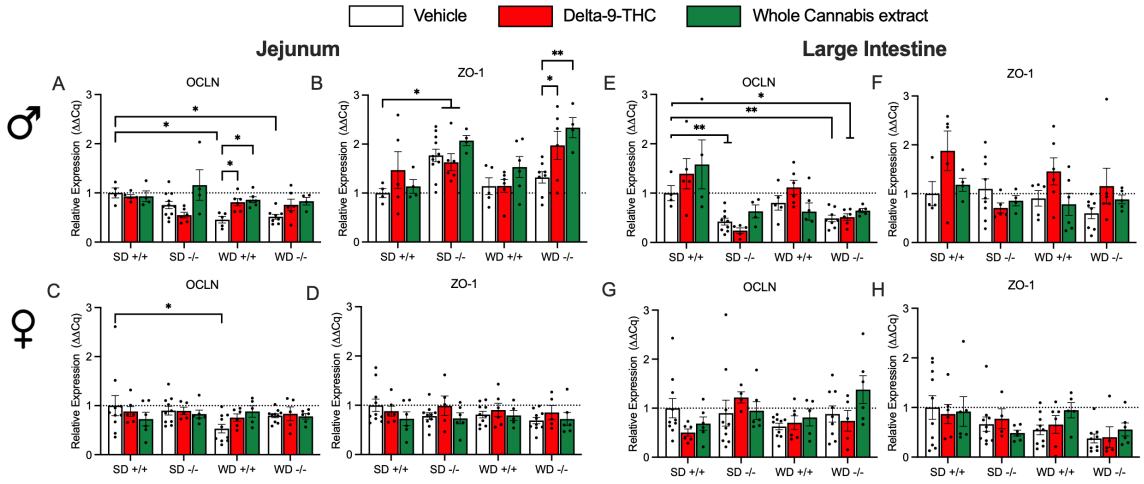


Figure 1. 4: Cannabinoids restore jejunal tight junction gene expression in male diet-induced obesity

All gene expression is normalized to SD intCB1R+/+ vehicle controls within each sex. **(A)** Jejunal OCLN expression in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,57) = 1.427$, $p = 0.2372$), a significant effect of drug ($F(2,57) = 6.634$, $p = 0.0026$), and a significant effect of diet ($F(1,57) = 9.463$, $p = 0.0032$). A significant drug x diet interaction was observed ($F(2,57) = 5.112$, $p = 0.0091$). n = 4-11 per group. **(B)** Jejunal ZO-1 expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 27.51$, $p < 0.0001$), a significant effect of drug ($F(2,57) = 5.112$, $p = 0.0091$), and no effect of diet ($F(1,57) = 0.2831$, $p = 0.5967$). n = 4-9 per group. **(C)** Jejunal OCLN expression in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,73) = 0.3414$, $p = 0.5608$), no effect of drug ($F(2,73) = 0.1002$, $p = 0.9048$), and no effect of diet ($F(1,73) = 2.217$, $p = 0.1408$). n = 4-10 per group. **(D)** Jejunal ZO-1 expression in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,73) = 1.534$, $p = 0.2195$), no effect of drug ($F(2,73) = 1.054$, $p = 0.3539$), and no effect of diet ($F(1,73) = 1.483$, $p = 0.2272$). n = 4-10 per group. **(E)** Large intestine OCLN expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,55) = 37.81$, $p < 0.0001$), no effect of drug ($F(2,55) = 1.428$, $p = 0.2485$), and no effect of diet ($F(1,55) = 3.421$, $p = 0.0697$). A significant genotype x diet interaction was observed ($F(1,55) = 9.443$, $p = 0.0033$). n = 4-11 per group. **(F)** Large intestine ZO-1 expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,55) = 5.203$, $p = 0.0264$), a significant effect of drug ($F(2,55) = 3.557$, $p = 0.0353$), and no effect of diet ($F(1,55) = 1.268$, $p = 0.2651$). n = 4-9 per group. **(G)** Large intestine OCLN expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,71) = 6.088$, $p = 0.0160$), no effect of drug ($F(2,71) = 0.5837$, $p = 0.5605$), and no effect of diet ($F(1,71) = 0.02462$, $p = 0.8758$). n = 4-10 per group. **(H)** Large intestine ZO-1 expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,71) = 6.722$, $p = 0.0116$), no effect of drug ($F(2,71) = 0.2042$, $p = 0.8158$), and no effect of diet ($F(1,71) = 3.572$, $p = 0.0628$). n = 4-10 per group. Data are presented as mean ± SEM. Statistical analyses were performed using three-way ANOVA followed by Holm-Sidak post hoc tests.

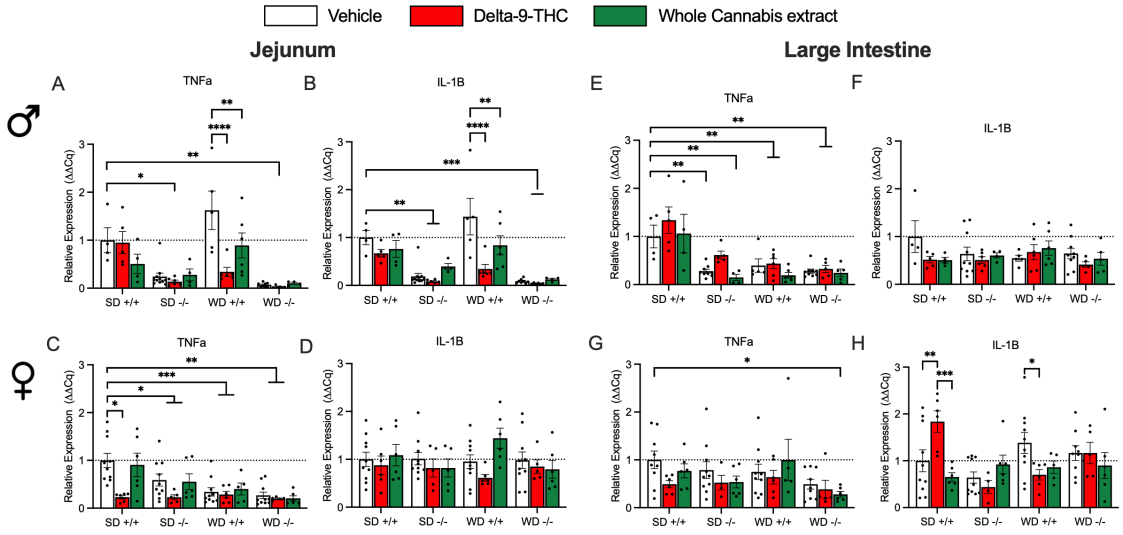

Vehicle
Delta-9-THC
Whole Cannabis extract
Jejunum
Large Intestine
♂
♀
A
B
C
D
E
F
G
H
TNFa
IL-1B
Relative Expression (ΔΔCq)
SD +/+
SD -/-
WD +/+
WD -/-

Figure 1. 5: Cannabinoids restore inflammatory homeostasis in a sex-dependent and region-specific manner during diet-induced obesity

All gene expression is normalized to SD intCB1R+/+ vehicle controls within each sex. **(A)** Jejunal TNFα expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 57.16$, $p < 0.0001$), a significant effect of drug ($F(2,57) = 5.766$, $p = 0.0052$), and no effect of diet ($F(1,57) = 0.008333$, $p = 0.9276$). Significant interactions were observed for drug x genotype ($F(2,57) = 4.747$, $p = 0.0124$), drug x diet ($F(2,57) = 3.578$, $p = 0.0344$), and drug x genotype x diet ($F(2,57) = 4.440$, $p = 0.0161$). n = 4-11 per group. **(B)** Jejunal IL-1β expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 75.66$, $p < 0.0001$), a significant effect of drug ($F(2,57) = 9.359$, $p = 0.0003$), and no effect of diet ($F(1,57) = 0.2277$, $p = 0.6350$). A significant drug x genotype interaction was observed ($F(2,57) = 6.845$, $p = 0.0022$). n = 4-9 per group. **(C)** Jejunal TNFα expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,73) = 6.326$, $p = 0.0141$), a significant effect of drug ($F(2,73) = 6.891$, $p = 0.0018$), and a significant effect of diet ($F(1,73) = 16.49$, $p = 0.0001$). A significant drug x diet interaction was observed ($F(2,73) = 4.380$, $p = 0.0160$). n = 4-10 per group. **(D)** Jejunal IL-1β expression in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,73) = 1.308$, $p = 0.2564$), no effect of drug ($F(2,73) = 1.913$, $p = 0.1549$), and no effect of diet ($F(1,73) = 0.0007303$, $p = 0.9785$). n = 4-10 per group. **(E)** Large intestine TNFα expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,55) = 27.49$, $p < 0.0001$), a significant effect of drug ($F(2,55) = 3.758$, $p = 0.0295$), and a significant effect of diet ($F(1,55) = 28.14$, $p < 0.0001$). A significant genotype x diet interaction was observed ($F(1,55) = 20.30$, $p < 0.0001$). n = 4-11 per group. **(F)** Large intestine IL-1β expression in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,57) = 1.719$, $p = 0.1951$), no effect of drug ($F(2,57) = 1.695$, $p = 0.1927$), and no effect of diet ($F(1,57) = 0.1500$, $p = 0.6999$). n = 4-9 per group. **(G)** Large intestine TNFα expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,70) = 6.592$, $p = 0.0124$), no effect of drug ($F(2,70) = 1.916$, $p = 0.1548$), and no effect of diet ($F(1,70) = 0.7917$, $p = 0.3766$). n = 4-10 per group. **(H)** Large intestine IL-1β expression in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,71) = 2.380$, $p = 0.1274$), no effect of drug ($F(2,71) = 0.7178$, $p = 0.4913$), and no effect of diet ($F(1,71) = 0.7216$, $p = 0.3985$). A significant genotype x diet interaction was observed ($F(1,71) = 4.218$, $p = 0.0437$), and a significant drug x genotype x diet interaction was observed ($F(2,71) = 6.047$, $p = 0.0038$). n = 4-10 per group. Data are presented as mean ± SEM. Statistical analyses were performed using three-way ANOVA followed by Holm-Sidak post hoc tests.

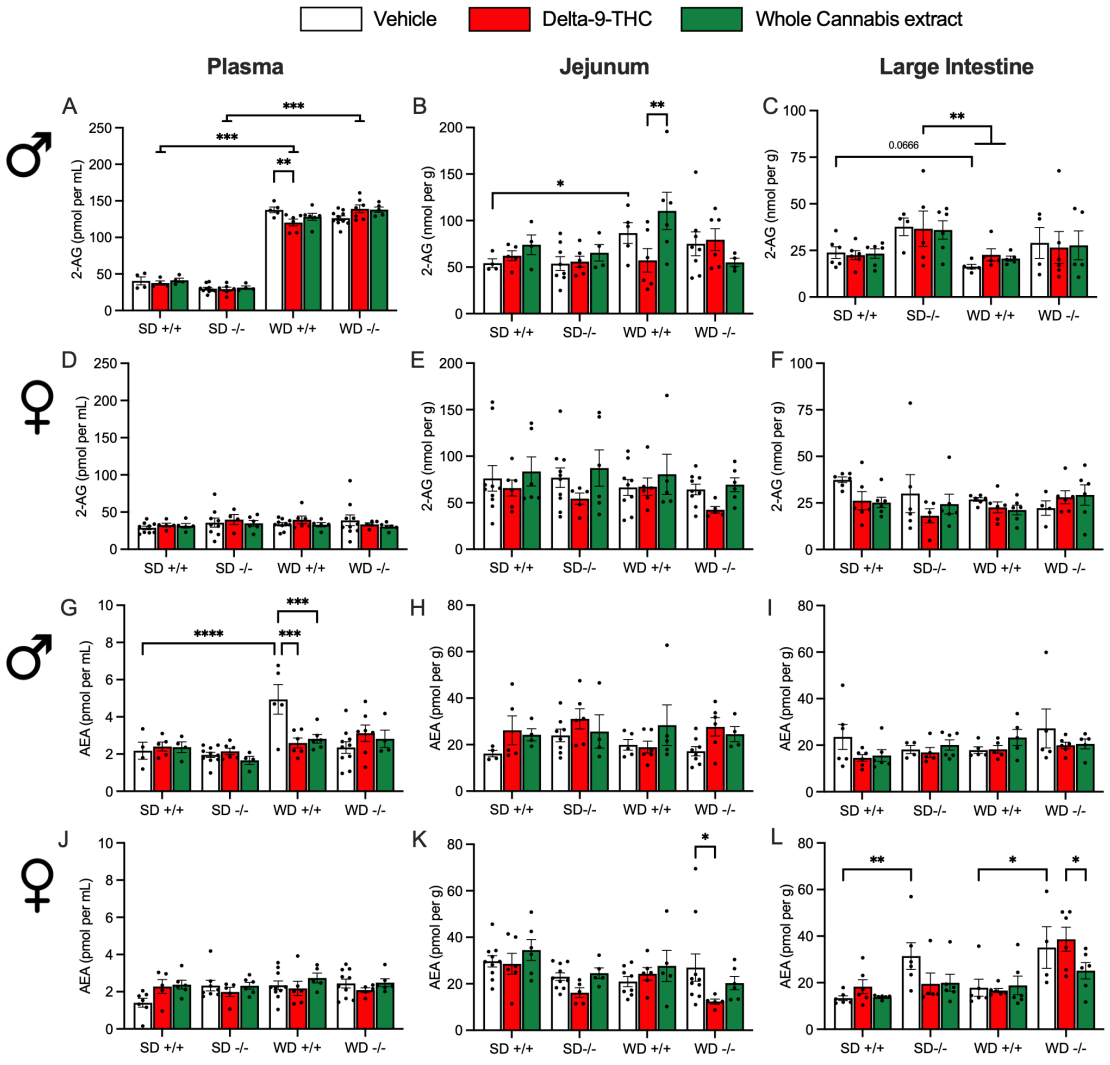

Vehicle
Delta-9-THC
Whole Cannabis extract
Plasma
Jejunum
Large Intestine
A
B
C
D
E
F
G
H
I
J
K
L
2-AG (pmol per mL)
2-AG (nmol per g)
AEA (pmol per mL)
AEA (pmol per g)
SD +/+
SD -/-
WD +/+
WD -/-
0.0666

Figure 1. 6: Diet-induced obesity drives region-specific and sex-dependent dysregulation of the endocannabinoid system

(A) Plasma 2-AG levels in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,59) = 0.7046$, $p = 0.4046$), no effect of drug ($F(2,59) = 0.6139$, $p = 0.5446$), and a significant effect of diet ($F(1,59) = 174.2$, $p < 0.0001$). Significant interactions were observed for genotype x diet ($F(1,59) = 11.09$, $p = 0.0015$) and drug x genotype x diet ($F(2,59) = 3.467$, $p = 0.0377$). n = 4-10 per group. **(B)** Jejunal 2-AG levels in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,54) = 2.106$, $p = 0.1525$), no effect of drug ($F(2,54) = 1.074$, $p = 0.3490$), and a significant effect of diet ($F(1,54) = 5.626$, $p = 0.0213$). n = 4-10 per group. **(C)** Large intestine 2-AG levels in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,51) = 11.36$, $p = 0.0014$), no effect of drug ($F(2,51) = 0.004338$, $p = 0.9957$), and no effect of diet ($F(1,51) = 3.770$, $p = 0.0577$). n = 5-6 per group. **(D)** Plasma 2-AG levels in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,68) = 0.6422$, $p = 0.4257$), no effect of drug ($F(2,68) = 0.4989$, $p = 0.6094$), and no effect of diet ($F(1,68) = 0.08841$, $p = 0.7671$). n = 4-10 per group. **(E)** Jejunal 2-AG levels in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,71) = 2.965$, $p = 0.0580$), no effect of drug ($F(2,71) = 1.115$, $p = 0.2945$), and no effect of diet ($F(1,71) = 1.587$, $p = 0.2118$). n = 4-10 per group. **(F)** Large intestine 2-AG levels in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,57) = 0.2136$, $p = 0.6457$), no effect of drug ($F(2,57) = 1.368$, $p = 0.2628$), and no effect of diet ($F(1,57) = 0.4649$, $p = 0.4981$). n = 5-6 per group. **(G)** Plasma AEA levels in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,60) = 0.0002556$, $p = 0.9873$), no effect of drug ($F(2,60) = 2.475$, $p = 0.0927$), and no effect of diet ($F(1,60) = 3.417$, $p = 0.0695$). Significant interactions were observed for drug x genotype ($F(2,60) = 10.47$, $p = 0.0001$), drug x diet ($F(2,60) = 8.129$, $p = 0.0008$), genotype x diet ($F(1,60) = 8.319$, $p = 0.0054$), and drug x genotype x diet ($F(2,60) = 5.351$, $p = 0.0073$). n = 4-10 per group. **(H)** Jejunal AEA levels in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,53) = 1.177$, $p = 0.2828$), a significant effect of drug ($F(2,53) = 3.211$, $p = 0.0483$), and no effect of diet ($F(1,53) = 0.5086$, $p = 0.4789$). n = 4-8 per group. **(I)** Large intestine AEA levels in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,52) = 0.6664$, $p = 0.4180$), no effect of drug ($F(2,52) = 1.536$, $p = 0.2248$), and no effect of diet ($F(1,52) = 2.323$, $p = 0.1335$). n = 5-6 per group. **(J)** Plasma AEA levels in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,67) = 0.1203$, $p = 0.7298$), no effect of drug ($F(2,67) = 2.137$, $p = 0.1260$), and no effect of diet ($F(1,67) = 2.737$, $p = 0.1027$). n = 4-10 per group. **(K)** Jejunal AEA levels in female mice. Three-way ANOVA revealed no effect of genotype ($F(1,71) = 2.974$, $p = 0.0890$), no effect of drug ($F(2,71) = 3.113$, $p = 0.0506$), and a significant effect of diet ($F(1,71) = 4.411$, $p = 0.0393$). n = 4-10 per group. **(L)** Large intestine AEA levels in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 26.43$, $p < 0.0001$), no effect of drug ($F(2,57) = 1.738$, $p = 0.1851$), and a significant effect of diet ($F(1,57) = 6.717$, $p = 0.0121$). n = 5-6 per group. Data are presented as mean ± SEM. Statistical analyses were performed using three-way ANOVA followed by Holm-Sidak post hoc tests.

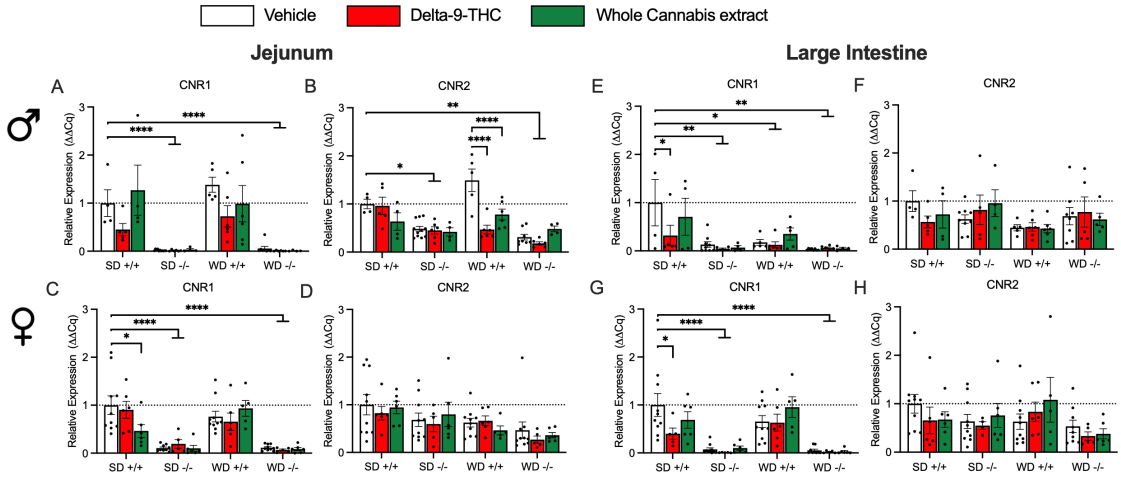
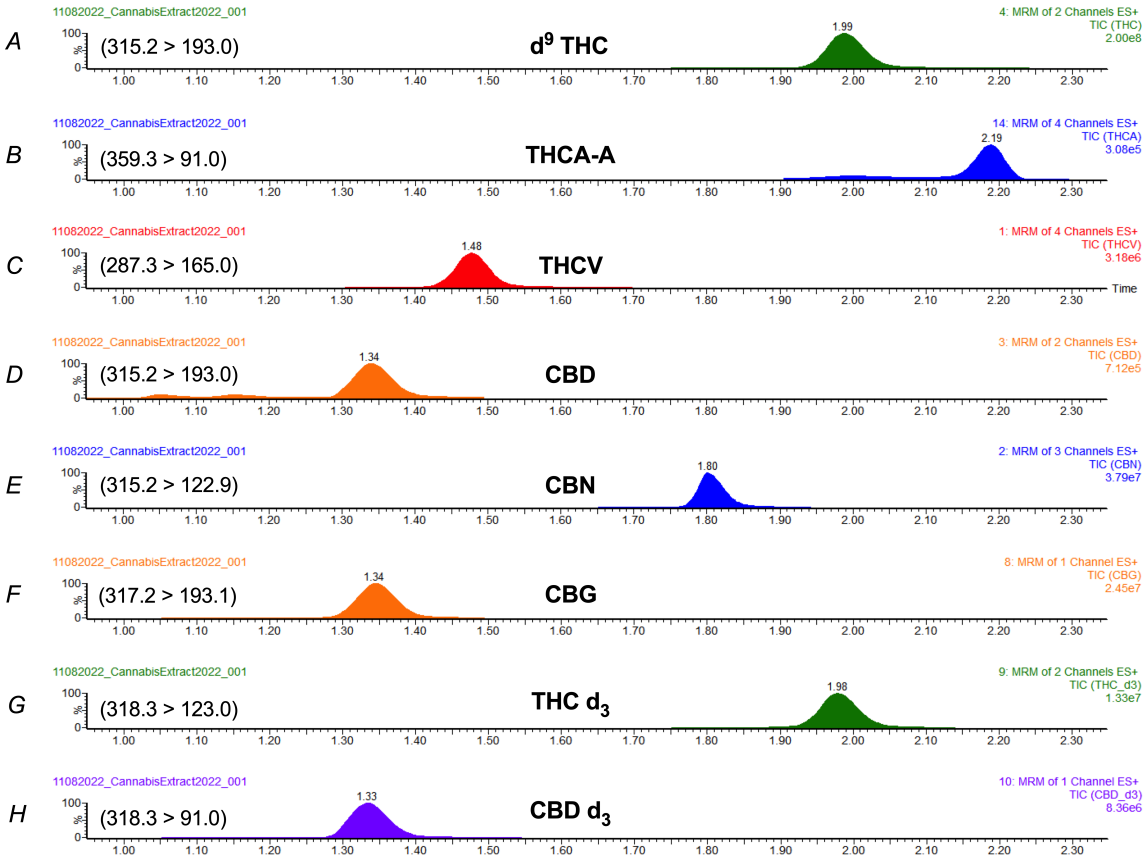

Vehicle
Delta-9-THC
Whole Cannabis extract
Jejunum
Large Intestine
A
CNR1
B
CNR2
E
CNR1
F
CNR2
C
CNR1
D
CNR2
G
CNR1
H
CNR2
Relative Expression (ΔΔCq)
SD +/+
SD -/-
WD +/+
WD -/-

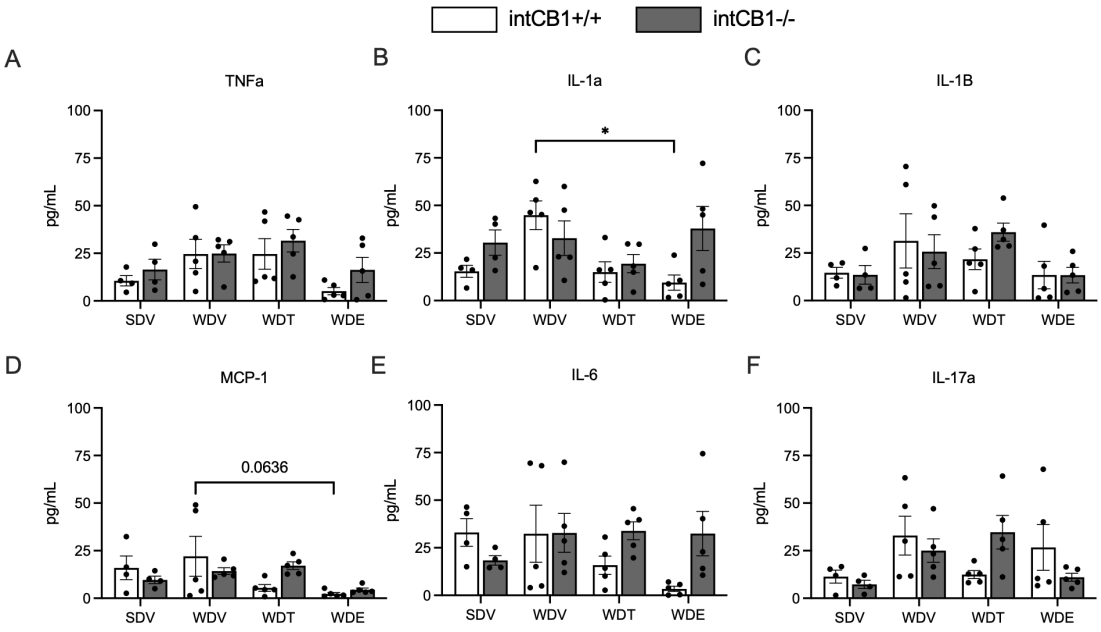
Figure 1. 7: Cannabinoid receptor expression in the intestine exhibits region-specific and sexually dimorphic regulation during diet-induced obesity

All gene expression is normalized to SD intCB1R+/+ vehicle controls within each sex. (A) Jejunal CNR1 expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 74.00$, $p < 0.0001$), a significant effect of drug ($F(2,57) = 3.475$, $p = 0.0377$), and no effect of diet ($F(1,57) = 0.3432$, $p = 0.5603$). n = 4-11 per group. **(B)** Jejunal CNR2 expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,57) = 60.93$, $p < 0.0001$), a significant effect of drug ($F(2,57) = 9.175$, $p = 0.0004$), and no effect of diet ($F(1,57) = 0.3664$, $p = 0.5473$). Significant interactions were observed for drug x genotype ($F(2,57) = 7.978$, $p = 0.0009$), drug x diet ($F(2,57) = 7.614$, $p = 0.0012$), and drug x genotype x diet ($F(2,57) = 4.519$, $p = 0.0151$). n = 4-9 per group. **(C)** Jejunal CNR1 expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,72) = 81.51$, $p < 0.0001$), no effect of drug ($F(2,72) = 0.6598$, $p = 0.5201$), and no effect of diet ($F(1,72) = 0.1109$, $p = 0.7401$). n = 4-10 per group. **(D)** Jejunal CNR2 expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,73) = 5.275$, $p = 0.0245$), no effect of drug ($F(2,73) = 0.4339$, $p = 0.6496$), and a significant effect of diet ($F(1,73) = 11.61$, $p = 0.0011$). n = 4-10 per group. **(E)** Large intestine CNR1 expression in male mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,53) = 18.80$, $p < 0.0001$), no effect of drug ($F(2,53) = 2.017$, $p = 0.1431$), and a significant effect of diet ($F(1,53) = 7.805$, $p = 0.0072$). A significant genotype x diet interaction was observed ($F(1,53) = 5.823$, $p = 0.0193$). n = 4-11 per group. **(F)** Large intestine CNR2 expression in male mice. Three-way ANOVA revealed no effect of genotype ($F(1,54) = 1.699$, $p = 0.1980$), no effect of drug ($F(2,54) = 0.03770$, $p = 0.9630$), and no effect of diet ($F(1,54) = 3.806$, $p = 0.0563$). n = 4-9 per group. **(G)** Large intestine CNR1 expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,71) = 63.22$, $p < 0.0001$), no effect of drug ($F(2,71) = 1.820$, $p = 0.1696$), and no effect of diet ($F(1,71) = 0.02073$, $p = 0.8859$). n = 4–10 per group. **(H)** Large intestine CNR2 expression in female mice. Three-way ANOVA revealed a significant effect of genotype ($F(1,71) = 5.418$, $p = 0.0228$), no effect of drug ($F(2,71) = 0.3870$, $p = 0.6805$), and no effect of diet ($F(1,71) = 0.4403$, $p = 0.5091$). n = 4-10 per group. Data are presented as mean ± SEM. Statistical analyses were performed using three-way ANOVA followed by Holm-Sidak post hoc tests.



Supplemental Figure 1. 1: Chromatograms for phytocannabinoids detected and quantified in whole-cannabis extract.

(A) delta 9-tetrahydrocannabinol (d9THC), **(B)** tetrahydrocannabinolic acid A (THCAA), **(C)** tetrahydrocannabivarin (THCV), **(D)** cannabidiol (CBD), **(E)** cannabinol (CBN), **(F)** cannabigerol (CBG), **(G)** deuterated delta 9-tetrahydrocannabinol (THCd3; internal standard), **(H)** cannabidiol (CBDd3, internal standard) (adapted from Avalos, Olmos et.al 2026).



Supplemental Figure 1. 2: Limited Systemic Inflammatory Response

Multiplex cytokine analysis was performed on plasma collected from male mice to assess the effects of genotype and cannabinoid treatment on systemic inflammatory markers. **(A)** TNFα levels. Two-way ANOVA revealed no significant interaction between genotype and drug treatment ($F(3,30) = 0.301$, $p = 0.824$), no main effect of genotype ($F(1,30) = 2.08$, $p = 0.160$), and a significant main effect of drug ($F(3,30) = 4.18$, $p = 0.0138$). **(B)** IL-1α levels. Two-way ANOVA revealed a trend toward interaction ($F(3,30) = 2.92$, $p = 0.0503$), no significant main effect of genotype ($F(1,30) = 3.03$, $p = 0.0918$), and a significant main effect of drug ($F(3,30) = 3.40$, $p = 0.0302$). **(C)** IL-1β levels. Two-way ANOVA revealed no significant interaction ($F(3,30) = 0.654$, $p = 0.587$), no main effect of drug ($F(3,30) = 2.51$, $p = 0.0774$), and no main effect of genotype ($F(1,30) = 0.116$, $p = 0.735$). **(D)** MCP-1 levels. Two-way ANOVA revealed no significant interaction ($F(3,30) = 1.98$, $p = 0.139$), no main effect of genotype ($F(1,30) = 0.00029$, $p = 0.986$), and a significant main effect of drug ($F(3,30) = 3.79$, $p = 0.0204$). **(E)** IL-6 levels. Two-way ANOVA revealed no significant interaction ($F(3,30) = 2.29$, $p = 0.0990$), no main effect of drug ($F(3,30) = 0.980$, $p = 0.415$), and no main effect of genotype ($F(1,30) = 1.75$, $p = 0.196$). **(F)** IL-17A levels. Two-way ANOVA revealed no significant interaction ($F(3,30) = 2.66$, $p = 0.0658$), no main effect of drug ($F(3,30) = 2.41$, $p = 0.0863$), and no main effect of genotype ($F(1,30) = 0.0667$, $p = 0.798$). Data are presented as individual data points with summary statistics (mean ± SD), with n = 4 -5 per group.

General Discussion

Overview and Significance

Diet-induced obesity is increasingly recognized as a condition that extends beyond systemic metabolic disturbances to include significant changes in intestinal function. Although obesity is classically defined by excess adiposity, insulin resistance, and chronic low-grade inflammation, growing evidence indicates that the gastrointestinal tract plays a central role in shaping these outcomes. In particular, disruption of intestinal barrier integrity has emerged as a key mechanism linking Western diet consumption to metabolic endotoxemia, systemic inflammation, and the progression of metabolic disease [18-21]. In this dissertation, I demonstrate that intestinal CB1R signaling is a key regulator of gut barrier integrity during diet-induced obesity, functioning in a sex-dependent manner. Furthermore, cannabinoid exposure restores barrier function through both CB1-dependent and CB1-independent mechanisms, in a sex-dependent and region-specific manner, highlighting the complexity of endocannabinoid signaling within the gastrointestinal tract.

Intestinal CB1R Signaling

A central finding of this dissertation is that intestinal CB1R signaling exerts divergent effects on metabolic and barrier phenotypes. In male mice, deletion of intestinal epithelial CB1R attenuated body weight gain during Western diet feeding, consistent with prior work demonstrating that peripheral CB1R signaling promotes hyperphagia and energy storage [64]. However, this metabolic improvement occurred alongside increased intestinal permeability and elevated circulating LPS, indicating that intestinal CB1R

signaling is protective for epithelial barrier integrity despite its contribution to obesogenic processes. These results reveal an important physiological tradeoff: intestinal CB1R signaling promotes metabolic dysfunction while simultaneously preserving barrier function during diet-induced stress. This dual role highlights the importance of tissue-specific cannabinoid signaling and suggests that intestinal CB1R serves a protective function in maintaining epithelial homeostasis under conditions of metabolic challenge.

Sex-Dependent Regulation of Gut Barrier Function

A major contribution of this work is demonstrating that cannabinoid signaling regulates intestinal physiology in a strongly sex-dependent manner. Female mice lacking intestinal CB1R were resistant to Western diet-induced weight gain and did not exhibit the pronounced endotoxemia observed in males. Although WD-fed female intCB1R+/+ mice exhibited increased permeability to 4 kDa FITC-dextran, this did not translate into elevated circulating LPS levels, suggesting that the degree or nature of barrier disruption differs between sexes. One potential explanation is the size-selective nature of permeability changes observed in females. FITC-dextran is substantially smaller than bacterial LPS complexes, which can reach molecular weights of approximately 60 kDa or greater depending on aggregation state and binding proteins [116]. Thus, Western diet exposure in females may induce relatively modest leak-pathway dysfunction sufficient to permit passage of smaller solutes, such as FITC-dextran, while still restricting the translocation of larger endotoxin-associated complexes into the circulation. In contrast, male mice exhibited both increased FITC-dextran permeability and elevated circulating

LPS, suggesting more severe or structurally distinct barrier disruption during diet-induced obesity.

These findings further suggest that females may possess compensatory protective mechanisms that limit progression from mild permeability defects to overt metabolic endotoxemia. Sex hormones likely play a central role in this protection. In particular, progesterone has been shown to exert anti-inflammatory and barrier-protective effects within the gastrointestinal tract, including modulation of epithelial tight junction stability, suppression of inflammatory cytokine production, and maintenance of mucosal integrity [106-109] . Together with estrogen signaling, progesterone may help preserve epithelial organization and reduce susceptibility to severe endotoxin translocation during metabolic stress. This interpretation is consistent with extensive evidence demonstrating sex differences in immune function, hormonal regulation, and endocannabinoid signaling [22, 80, 92]. Furthermore, recent studies have shown that epithelial-specific estrogen receptor signaling can produce opposing effects in males and females, reinforcing the concept that intestinal epithelial pathways themselves are intrinsically sex-dependent [24].

In addition, female mice showed limited responsiveness to cannabinoid treatment across metabolic and inflammatory outcomes, suggesting that females may rely on regulatory pathways that are less dependent on epithelial CB1R signaling to maintain intestinal homeostasis. Together, these findings establish sex as a primary determinant of intestinal barrier regulation and cannabinoid responsiveness. Importantly, they highlight the need to include both sexes in studies of metabolic disease and gut physiology, as mechanisms identified in males may not directly translate to females.

Cannabinoid-Mediated Restoration of Barrier Function

This dissertation demonstrates that cannabinoid exposure improves intestinal barrier function through both CB1-dependent and CB1-independent mechanisms. Treatment with Δ^9-THC and whole cannabis extract reduced intestinal permeability and attenuated LPS translocation, particularly in male mice fed a Western diet. Notably, these protective effects were observed even in mice lacking epithelial CB1R, indicating that non-epithelial or alternative signaling pathways contribute to cannabinoid-mediated barrier restoration. Potential mechanisms include signaling through CB2 receptors, TRP channels, and PPARs, as well as indirect effects mediated by immune cells, enteric neurons, or the gut microbiota [48]. These findings highlight the complexity of cannabinoid signaling within the intestinal environment and suggest that barrier protection involves coordinated interactions across multiple cell types and signaling systems.

Tight Junction Regulation and Context-Dependent Effects

Although cannabinoids improved functional measures of barrier integrity, their effects on tight junction and inflammatory gene expression were more context-dependent. In the jejunum of Western diet-fed male intCB1R+/+ mice, cannabinoid treatment restored occludin expression and normalized pro-inflammatory cytokines. In contrast, these effects were diminished in intCB1R-/- mice, indicating that epithelial CB1R signaling is required for full transcriptional recovery of tight junction-associated pathways. These findings suggest that functional improvements in permeability can occur independently of complete molecular restoration of epithelial gene expression. Thus,

cannabinoid signaling may influence barrier integrity through multiple mechanisms, including modulation of tight junction structure, epithelial repair processes, immune signaling, and luminal factors. Interestingly, male intCB1R-/- mice exhibited increased permeability and endotoxemia despite reduced local expression of inflammatory genes. This dissociation indicates that barrier dysfunction in this model is not solely driven by inflammation but may instead reflect structural alterations in epithelial integrity, particularly within tight junction complexes. These results support a model in which CB1R contributes directly to maintaining epithelial structure during metabolic stress.

Regional Specialization of Intestinal Barrier Regulation

The effects of cannabinoid signaling on barrier function were strongly region-specific, with the most pronounced changes occurring in the jejunum. This pattern aligns with the small intestine's unique role in nutrient sensing and endocannabinoid signaling. In contrast, the large intestine is characterized by dense microbial populations and depends heavily on a structured mucus barrier to maintain separation between luminal contents from the epithelium. The small intestine primarily relies on epithelial defenses, including antimicrobial peptides, whereas the colon relies on a multilayered mucus system composed of polymerized MUC2 [21, 95, 96]. These fundamental differences in barrier architecture likely underlie the distinct responses observed across intestinal regions. The present findings therefore reinforce the concept that intestinal barrier regulation is regionally specialized and shaped by local physiological context.

Dysregulation of the Endocannabinoid System in Obesity

This study further demonstrates that diet-induced obesity causes significant dysregulation of the endocannabinoid system in a sex- and region-specific manner. In male mice, Western diet feeding increased plasma and jejunal levels of 2-AG and AEA, while reducing 2-AG levels in the large intestine. In contrast, female mice showed minimal changes in endocannabinoid tone. These findings support the concept that obesity is associated with elevated endocannabinoid signaling, particularly in tissues involved in energy balance [45, 46]. However, the observed reduction in colonic 2-AG suggests a loss of protective signaling within the distal intestine, which may contribute to barrier dysfunction. Together, these results highlight a complex, region-specific pattern of endocannabinoid dysregulation that may differentially influence metabolic and intestinal outcomes.

Cannabis Use and Human Health

The findings of this dissertation are particularly relevant amid rising cannabis use. As legalization expands and cannabis consumption becomes more widespread, understanding its physiological effects on metabolic health and intestinal function is increasingly important. Cannabis contains multiple bioactive compounds, including Δ^9-THC and CBD, which can exert distinct and sometimes complementary effects. The observation that whole cannabis extract often produces equal or greater effects than Δ^9-THC alone supports the concept of an "entourage effect," in which multiple phytocannabinoids contribute to physiological outcomes. CBD, in particular, has been shown to reduce inflammation and improve epithelial barrier function through

mechanisms that do not rely on direct CB1 activation[112-115, 117]. These findings suggest that real-world cannabis exposure may have complex, context-dependent effects on gut barrier integrity.

Intestinal Alkaline Phosphatase as a Potential Mechanism

An additional mechanism that may contribute to cannabinoid-mediated barrier regulation is IAP, an enzyme that detoxifies LPS and maintains epithelial homeostasis. Reduced IAP activity has been linked to obesity, metabolic endotoxemia, and inflammation (Field [75, 76]. Although IAP was not directly measured in this study, loss of intestinal CB1 receptors significantly increased circulating LPS levels. Additionally, in male intCB1+/+ mice, a reduction in circulating LPS following cannabinoid treatment suggests a potential interaction between cannabinoid signaling and IAP function. Supporting this possibility, human studies have demonstrated that obese individuals exhibit increased circulating AEA alongside reduced duodenal expression of IAP and the tight junction protein ZO-1. Furthermore, plasma AEA levels are negatively associated with both IAP and ZO-1 expression, suggesting a link between endocannabinoid dysregulation and impaired barrier function [78]. These findings raise the possibility that cannabinoid signaling may influence intestinal barrier integrity, in part, through modulation of IAP activity.

Future Directions

Several important directions for future research emerge from this work. First, refining intestinal permeability measurements using earlier time points following FITC-dextran administration will enable more precise assessment of regional barrier function. Second, the role of sex hormones in regulating endocannabinoid signaling and barrier integrity should be directly examined using hormonal manipulation. Third, the contribution of CB2 receptor signaling to intestinal barrier regulation warrants investigation using targeted genetic models. Fourth, future studies should evaluate the relationship between cannabinoid signaling and IAP expression or activity. Finally, extending cannabinoid treatment paradigms to longer durations will help determine whether sustained improvements in metabolic health are associated with long-term restoration of intestinal barrier function.

Conclusions

In conclusion, this dissertation demonstrates that intestinal cannabinoid signaling is a critical regulator of gut barrier function during diet-induced obesity. These effects are highly sex-dependent, region-specific, and mechanistically complex, involving both CB1R-dependent and CB1R-independent pathways. The findings establish the endocannabinoid system as a key component of the gut–metabolic axis and provide a foundation for future studies aimed at targeting cannabinoid signaling to improve intestinal barrier integrity and metabolic health.

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