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THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE REGULATION OF VASCULAR TONE

PhD thesis

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LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
α-SMA	α- smooth muscle actin
AA	Arachidonic acid
ABHD6	alpha/beta-Hydrolase domain containing 6
Ach	Acetylcholine
AEA	Anandamide / N-arachidonoylethanolamine
Ang II	Angiotensin II
ANOVA	Analysis of variance
ADP	Adenosine-diphosphate
CBD	Cannabidiol
CVD	Cardiovascular disease
COX-2	Cyclooxigenase-2
cGMP	Cyclic guanosine monophosphate
C57BL6	Black 6 type mouse
CB- 1R	Cannabinoid receptor type 1
CB- 1R +/+	Cannabinoid receptor 1 - wild type mice
CB- 1R -/-	Cannabinoid receptor 1 - knock out mice
CB- 2R	Cannabinoid receptor type 2
CCL-2	Monocyte chemoattractant protein-1
DAB	3,3' diaminobenzidine
DAG	1,2 diacylglycerol
DAGL	diacylglycerol lipase
DNA	Deoxyribonucleic acid
DP1-2	PGD receptor
EA	ethanolamide
eCB	Endothelial cannabinoid receptor
ECS	Endocannabinoid system
EET-EA	Epoxyeicosatrienoic Acid Ethanolamid
E2	Estradiol
EP 1-4	Prostaglandine E2 receptors
ER-α	Estrogen receptor alpha

ER-β	Estrogen receptor beta
eNOS	Endothelial nitric oxide synthase
FAAH	Fatty acid amid hydrolase
FSH	Follicle-stimulating hormone
FP / FPalt4	PGF receptor / variant
G	glycerol
GPCR	G protein coupled receptor
GPR18 / 30 / 55 / 119	G protein coupled „orphan receptors”
GnRH	Gonadotropin-releasing hormone
HRP	Horseradish peroxidase
HE	Hematoxylin eosin
HETE-EA	Hydroxyeicosatetraenoic Ethanolamid
HNE	4- hydroxynonenal
HPO	Hypothalamo – pituitary – ovarian axis
IFN-γ	Interferon gamma
IL-6	Interleukine-6
IL-10	Interleukine-10
IP	prostaglandin receptor
KO	Knock out genotype (-/-)
LH	Luteinizing hormone
LPS	Lipopolysaccharide
NAAA	N-acylethanolamine acid amide hydrolase
NAD	Nicotinamide adenine dinucleotide
NAPE-PLD	N-acyl phosphatidylethanolamine -phospholipase D
NF-κB	Nuclear factor kappa-light-chain-enhancer activated B cells
NO	Nitric oxide
NT	3- nitrotyrosine
MDA	Malonyl dialdehyde assay
MAPK / PI3K	Mitogen activated protein kinase
	Phosphoinositide 3-kinase
MAG	Monoacylglicerol
MAGL	Monoacylglicerol lipase

MMP-9	Matrix metalloproteinase 9
O.D.	Optical density
PAR	Poly (ADP-ribose)
PARP-1	Poly (ADP-ribose) polimerase 1
PCOS	Polycystic ovarian syndrome
PFA	Paraformaldehyde
PPARγ	Peroxisome proliferator-activated receptor γ
PGDS / PGES / PGFS	Prostaglandin synthase isoforms
PGI2	prostacyclin / prostaglandin I2
PGD2 / PGE2	Prostaglandin isoforms
PGF2 / PGH2	Prostaglandin isoforms
PLA2	Phospholipase A2
RF	Resorcin fuchsin
SEM	Standard error of mean
TBXAS	Tromboxane A2 synthase
THC	Delta 9 tetrahydrocanabinol
TNF -α-	Tumor necrosis factor alpha -
TLR-4-	Toll like receptor 4
TRPV-	Transient receptor potential vallinoid receptor -
TXA2	Tromboxane A2
IP / DP	Prostacyclin receptor I / DP
TP	Tromboxane receptor
VSMC	Vascular smooth muscle cell
WT	Wild type genotype (+/+)

1. INTRODUCTION

The cannabinoid system is named after the psychoactive substance found in the hemp plant (Cannabis sativa), delta 9 tetrahydrocannabinol (THC). This substance acts predominantly on the body's CB-1 cannabinoid receptor, which was discovered in the 1990s (1). Shortly afterwards, endogenous ligands of this receptor were discovered, such as anandamide (AEA) and 2−arachidonoylglycerol (2-AG) (2). A few years later, another cannabinoid receptor was identified, the Cannabinoid receptor 2 (CB-2) (1).

1.1. The endocannabinoid system

Nowadays, the endocannabinoid system (ECS) is mainly understood as the signaling mediated by a variety of endocannabinoids (3) on the CB-1 and CB-2 cannabinoid receptors. The most well-known members are AEA and 2-AG (lipid mediators). The ECS plays a crucial role in the regulation of mood and emotions, as well as in the mediation of hunger and satiety, contributing to metabolic control (4, 5). AEA is involved in learning and memory function, affects synaptic plasticity (6), regulates appetite via orexinergic neural activity (7) and also regulates cell division, differentiation, and apoptosis (5).

In addition to the activation of endocannabinoid receptors, many endo− and exocannabinoids can also induce homeostatic effects through other mechanisms, for example by acting on Transient receptor potential vanilloid channels (TRPV) and the related ankyrin receptor (TRPA-1) or nuclear peroxisome proliferator receptors (PPARs) (8, 9).

1.1.1. Metabolism of endocannabinoids

AEA is produced through several enzymatic pathways, from its initial substrate N-acyl phosphatidylethanolamine (NAPE) synthesized in the cell membrane by Arachidonic acid trans-acylase. AEA synthesis can occur in a single step via the hydrolysis of NAPE, catalyzed by the enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), or in two steps involving phospholipase C (PLC) and phosphatase enzymes, or through multiple steps of deacylation and the cleavage of glycerophosphate (10, 11).

Degradation of anandamide is also possible through several enzymatic pathways, including hydrolysis by fatty acid amide hydrolase (FAAH) or N-acyl-ethanolamine amidase (NAAA) resulting in arachidonic acid, and ethanolamine. The oxidation of AEA

can result in the formation of several biologically active substances through various pathways: Anandamide is primarily oxidized by three enzyme families. Cyclooxygenase-2 (COX-2) converts AEA into various prostaglandin ethanolamides, especially under inflammatory conditions, while 12- and 15-lipoxygenases (LOX) generate hydroxylated AEA metabolites. Cytochrome P450 monooxygenases further degrade AEA via epoxidation — mainly by CYP3A4, CYP2D6, and CYP4X1 to form EET-EAs (Epoxyeicosatrienoic Acid Ethanolamides)— and by hydroxylation through CYP4F2 or CYP2D6 to yield 20-HETE-EA (Hydroxyeicosatetraenoic Ethanolamid). These P450-derived epoxy-AEA products are then hydrolyzed by microsomal and soluble epoxide hydrolases into dihydroxy derivatives, completing the oxidative breakdown of anandamide (12, 13) (See in Figure 1.).

The **synthesis of 2-AG** begins with diacylglycerol (DAG) synthetized from membrane phospholipids by the α and β isoforms of phospholipase C (PLC) and further cleaved by diacylglycerol lipase (DAG-L). Another possibility is through the activity of phospholipase A1 (PLA1) and lyso-PLC (14).

2-AG can be degraded through hydrolysis by monoacylglycerol lipase (MAG-L) that degrades 2-AG resulting in 85% decrease in 2-AG action, while other enzymes degrade the remaining 15% to arachidonic acid and glycerol (15) (See in Figure 1.).

Recent evidence also indicates that both AEA and 2-AG can be deactivated by COX-2. Research demonstrates that the oxidizing capacity of COX-2 is comparable to the degradation capacity of MAG-L in case of 2-AG, but significantly less for AEA (16-18) (See in Figure 1.).

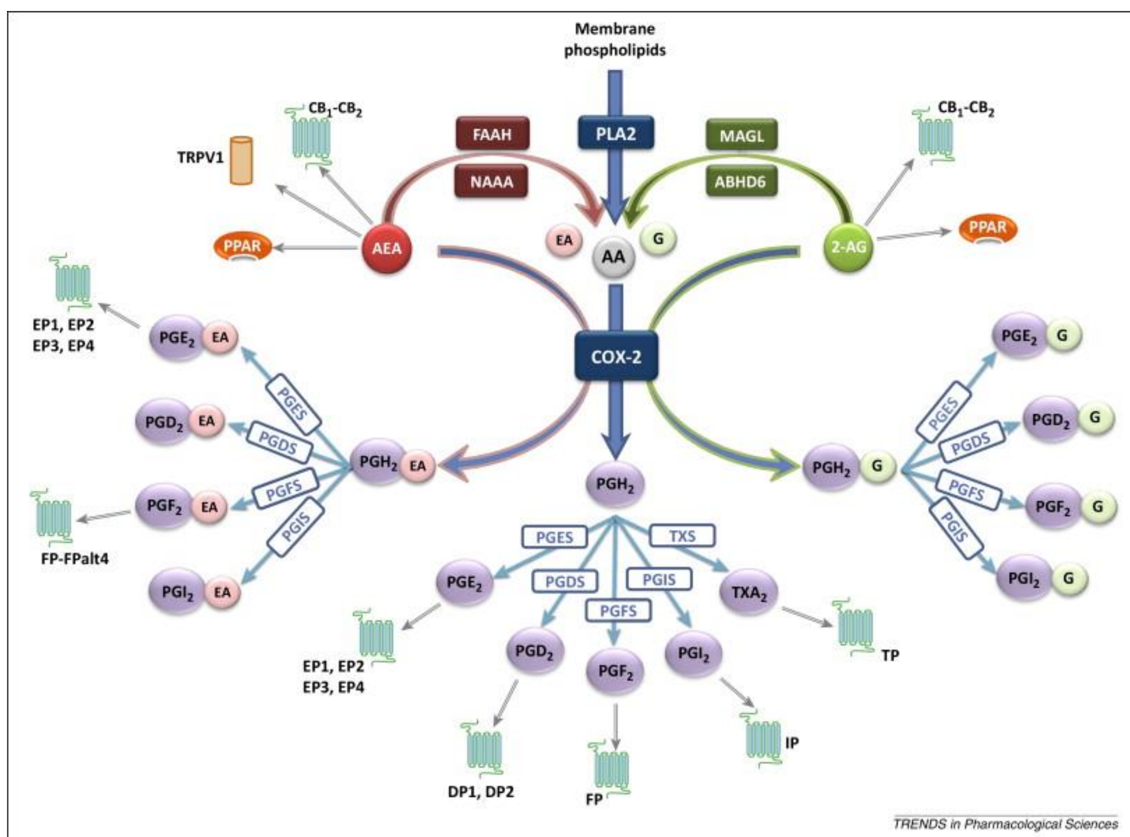

Figure 1 - Degradation of the endocannabinoids and the role of COX–2 in the metabolism (with the approval of the copyright owner) (16)

Endocannabinoids AEA and 2-AG can be degraded via hydrolysis, or oxidative pathways. AEA can be hydrolyzed by FAAH or NAAA producing arachidonic acid and ethanolamine. On the other hand, 2-AG can be hydrolyzed by MAGL or ABHD6 also to arachidonic acid glycerol. The key enzyme in the oxidative degradation of these endocannabinoids in both cases is COX-2 which produce prostaglandin ethanolamides from AEA and prostaglandin glycerol esters from 2-AG.

Abbreviations: PLA2 - Phospholipase A2; FAAH – fatty acid amide hydrolase; NAAA - N-acylethanolamine acid amide hydrolase; MAGL - Monoacylglycerol lipase; ABHD6 - alpha/beta-Hydrolase domain containing 6; AA – arachidonic acid, EA – ethanolamide; G – glycerol; AEA – anandamide; 2-AG – 2-arachidonoylglycerol; PPAR – peroxisome proliferator activator receptor; TRPV – transient receptor potential vanilloid receptor; EP1-4 - Prostaglandine E2 receptors; PGH2 – Prostaglandine H2; PGE2 – Prostaglandine E2; PGD2 – Prostaglandine D2; PGF2 – Prostaglandine F2; PGI2 – Prostaglandine I2; PGES – Prostaglandine E synthase; PGDS – Prostaglandine D synthase; PGFS – Prostaglandine F synthase; PGIS – Prostaglandine I synthase; TXA2 – thromboxane – A2; FP-FPalt4 – PGF receptor variant; TP – thromboxane receptor; IP – prostaglandin receptor; FP - PGF receptor; DP1-2 – PGD receptor;

1.2. Exocannabinoids

Exocannabinoids are all non-endogenous ligands that act on cannabinoid receptors (CB-1 and CB-2). They can be divided into two main groups — phytocannabinoids and synthetic cannabinoids. The former group are plant-derived active substances, predominantly isolated from the plant hemp, or in other name cannabis sativa; while synthetic cannabinoids are chemically produced targeted receptor agonists or antagonists (19).

The best known exogenous cannabinoid is THC, derived from cannabis sativa. THC is found not only in the cannabis sativa plant, but also in higher concentrations in Indian hemp (cannabis indica), also known as marijuana. THC is well known for its psychoactive effects via the CB-1 cannabinoid receptor. However, in addition to being a partial agonist of the CB-1 receptor, it also exerts a similar partial agonist effect on the CB-2 receptor (20-23). Both receptors are involved in signal transduction via the $G_{i/o}$ protein (they are G-protein coupled receptors: GPCR receptors) (24). Moreover, THC is capable of acting on other receptors such as the recently identified „orphan receptors" e.g.: GPR55, GPR 18, GPR 119, or the vanilloid receptors TRPV and the PPARγ receptor (22, 25).

Other active constituents of cannabis sativa can also modulate the endocannabinoid system – for example cannabidiol (CBD) which has a much broader spectrum of action. It has a dominant antagonistic — allosteric modulator effect on both CB-1 and CB-2 receptors, and can also increase AEA levels by inhibiting the enzyme FAAH (18). It also inhibits the GPR55 orphan receptor but has agonistic effects on TRPV1, and serotonin (5-HT1A) receptors (26).

Several other phytocannabinoids isolated from cannabis sativa are known but are less researched or toxic, such as cannabigerol, cannabiol, cannabitriol, Δ^8-THC (27).

Synthetic exocannabinoids generally have better receptor specificity and act predominantly on CB-1 and/or CB-2 receptors. The most well-known is the CB-1 receptor selective inverse agonist rimonabant (SR141716) (28, 29).

1.3. The cannabinoid system under healthy and pathological conditions

As mentioned earlier, the cannabinoid system plays an important regulatory role in homeostasis by modifying mood and metabolism, however; it also has immune and cardiovascular effects (30). The modification we can reach by utilizing various exocannabinoids may depend on the state of the homeostasis (physiological or diseased), the dose and the length of the treatment period (20, 31). For example, THC in low doses has beneficial effects in the treatment of hypertension, on the other hand, frequent, high-dose THC intake (e.g. in case of overdose) has cardiovascular side effects including heart attacks, stroke, or cannabis arteritis (32-34).
These findings collectively indicate that the endocannabinoid system may be Janus-faced upon the homeostasis of the organism.

1.3.1. Side effects of exocannabinoid use in healthy individuals

The adverse effects of cannabis or marijuana use for recreational purposes have been researched for a long time. It is well established that, in addition to the psychoactive effects, chronic cannabis use in high doses has systemic adverse effects (35). Its addictive potential is not outstanding among drugs, approximately the same as alcohol, being around 8-9% according to surveys (36, 37). Moreover, if pure Cannabidiol (CBD) oil with no psychoactive effects is consumed by users - addiction potential can be completely excluded (38). The increasing prevalence of synthetic cannabinoid derivatives, which produce more intense psychoactive effects than phytocannabinoids, is associated with an increased frequency of hospitalisation due to its psychosis-inducing ability and adverse effect profile previously described (39, 40). Short-term effects disturb cognitive function, but chronic use can lead to permanent cognitive dysfunction (41).
They also have notable acute and chronic cardiovascular side effects. During acute effect the heart rate is increased, and peripheral vasodilation may lead to unstable blood pressure and blood pressure fluctuations (42). In the presence of other tendencies, it may trigger myocardial infarction and stroke (43, 44).
In case of chronic consumption, increased cardiovascular risk is observed (34). Another severe complication is cannabis arteritis, which can even lead to the necrosis of the phalanges (45-47). In this case, patients experience increased platelet activation due to cannabinoids (48).

Cannabinoids not only act on the vessels but also act on the heart - in addition to the already known vasorelaxant effect - have a significant positive chronotropic effect, resulting in tachycardia. This effect of increased heart rate is also present in already abstinent chronic drug users (e.g. patient who used to smoke THC for 10 years in his or her life but abstinent for 15 years) in an abstinent state without cannabinoid influence (48). Chronic use leads to a sympathetic overactivation which may consequently lead to atrial fibrillation. In comparison to infrequent drug use and drug use lasting less than a week, chronic drug users had an approximately 15% higher risk to suffer a myocardial infarction (MI) (48).

While atherosclerosis is the most common complication of MI, it is important to investigate the intima-media ratio of blood vessels. Clinical studies have shown that rimonabant, known as an inverse agonist of the CB-1 receptor, does not significantly affect the structure of the vessel wall (49). In case of healthy, young, chronic cannabis (THC dominant) users after THC contained tobacco inhalation the arterial stiffness and the heart rate also elevated (50). Further research is needed to compare these results to the usage in pathological conditions.

1.3.2. Beneficial therapeutic effects in pathological conditions

Despite the predominantly adverse effects of THC in healthy recreational users, the number of clinical applications in pathological conditions demonstrate its beneficial effects (20).

In several countries, its analgesic effects are specifically employed in patients with chronic pain or terminal cancer that is resistant to therapy (51). However, not only its analgesic effect can be used in cancer therapy. Its effect on the immune response was found to be beneficial in testicular and breast cancer (22, 52).

On the other hand, cannabinoids have been demonstrated to impede the effect of antitumor immunotherapy (immune checkpoint inhibitors) by inhibiting the T cell specific immunity through the CB-2 receptor, influencing the JAK/STAT pathway. As a result, patients with higher AEA levels had worse overall survival. According to these results, cannabis consumption should be controlled in case of patients receiving immune-based antitumor therapy like immune checkpoint inhibitors, but it can be also a good choice for palliative therapy (53).

In case of cardiovascular diseases, the treatment with CBD demonstrated a favorable side effect profile based on rodent models: Chronic CBD treatment (10mg/kg daily for 2 weeks) tend to improve the Acetylcholine (Ach) induced endothelial dependent vasorelaxation (54).
Chronic low-dose THC treatment (0.15mg/ kg/day) in case of streptozotocin induced diabetic rats significantly reduced the blood glucose levels, and improved the vascular reactivity and left ventricular function (55, 56). In addition, based on several human and animal models, cannabinoids can significantly influence the endocrine hormone balance based on its impact on the energy homeostasis, appetite, and well-being (55, 56).

1.3.3. Cannabinoid effects on the cardiovascular system

There are several pharmacological target receptors for endo- and exocannabinoids which act on the vasculature or on the heart. The primarily examined cardiovascular-active cannabinoids are the endocannabinoid AEA, the phytocannabinoid THC, the synthetically modified CBD variant Abnormal Cannabidiol (Abn-CBD), and the synthetic analogue CP55940 and WIN 55-212 (57).
The literature suggests that cannabinoids have predominantly vasorelaxant effects (48).
The vasorelaxant effects of recreational cannabis use may include orthostatic hypotension in overdose, and rebound hypertension following drug degradation, especially in the case of synthetic exocannabinoids (58).
Cannabinoids are able to exert direct (through TRPV channels) or indirect vasorelaxant effects (via PPARγ). While the vanilloid receptor agonism mostly causes a Ca^{2+} signal (59), the latter PPARγ agonism causes metabolic effects (reduced atherosclerosis, reduced plasma triglyceride levels etc.) (60). The CB-2 receptor is dominantly responsible for immunomodulatory effects and thus has no direct vasoactive impact (9, 57).
It is important to note that, among several recognized receptors, both the endocannabinoid AEA and other exocannabinoids exert vasorelaxant effects directly via the endothelium. In study from 1999, Járai et. al. identified an isolated endothelial vasorelaxant cannabis effect in CB-1 and CB-2 knockout mice, which was eliminated only following endothelial layer separation or the administration of large doses of SR141716 (Rimonabant). It was proposed that a distinct endothelial cannabinoid receptor (eCB) exists alongside the

previously identified classical CB-1 and CB-2 receptors. Despite of the low research intensity in this area, there is significant therapeutic potential for modulating this receptor (61).

1.4. Endotoxin shock as a pathological condition

Endotoxemia and endotoxin shock result from excessive immunological activation, usually initiated by lipopolysaccharides (LPS) derived from Gram-negative bacteria. LPS molecules interact with Toll-like receptor 4 (TLR4) on immune cells, particularly macrophages and dendritic cells, triggering a signaling cascade through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen activated protein kinase (MAPK) pathways. This leads to a substantial release of pro-inflammatory cytokines, including Tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6), which contribute to systemic inflammation and endothelial dysfunction. Concurrently, the complement system undergoes hyperactivation, exacerbating inflammation and tissue injury via anaphylatoxins such as C3a and C5a. These molecular occurrences can lead to vascular permeability (endothelial dysfunction), hypotension, increased oxidative-nitrative stress, and multiorgan dysfunction typical of septic or endotoxin shock (62).

It has been observed that patients in septic shock have a mortality rate of 50% (63). The cardiovascular response can be divided into two distinct phases. The first phase is characterized by a hyperdynamic stage, during which blood pressure is maintained and cardiac output is elevated. The second phase is marked by the heart's inability to maintain the necessary cardiac output to sustain blood pressure within the dilated vasculature. In rodents with sepsis, cardiovascular dysfunction is observed, which is characterized by impaired contractility and endothelial dysfunction due to inflammation (64, 65). This inflammation leads to an increased production of oxygen and nitrogen-derived reactive species. Elevated oxidative and nitrative stress may lead to non-specific modifications of lipids and proteins, as well as single- and double-strand DNA breaks. As a result of single-strand DNA breaks, poly(ADP-ribose) polymerase 1 (PARP-1) is activated, which utilizes NAD^+ to build ADP-ribose polymers (PAR). Subsequently, PAR attaches to

histones to assist in repair mechanisms. PAR-ylation is a protein modification process that can have an impact on the cell's self-regulatory mechanisms, including DNA repair, gene transcription, cell death, cell metabolism, and cell cycle. It is important to note that it can also deplete NAD^+ and cause cellular energy failure. Additionally, the abundance of PAR can serve as a marker of DNA damage. Furthermore, reactive oxygen and nitrogen species, along with associated cellular damage, have been found to contribute to the development of cardiovascular diseases (66, 67).

1.4.1. Cannabinoid effects in inflammatory conditions

While the cardiovascular effects are mainly mediated by CB-1 receptor, the effects of cannabinoids on the immune system are predominantly mediated by CB-2 receptors. On the other hand, not only CB-2, but also CB-1 has been identified as a promising therapeutic target, for instance in the prevention of organ rejection. In fact, THC was recently discovered to attenuate the host-versus-graft response and delay the rejection of skin grafts in mice by reducing T cell proliferation and diminishing early stage rejection-indicator cytokines, such as IL-2 and interferon gamma (IFN-γ). Additionally, the authors demonstrated that these effects were mediated through the activation of CB-1 receptors and the induction of MDSCs (Myeloid derived supressor cells) by utilizing selective antagonists and CB-1 and CB-2 KO rodents (68).

Cannabinoids mainly have anti-inflammatory effects via the CB-2 receptors by affecting the ratio of pro- and anti-inflammatory cytokines. Stimulation of the CB-2 receptor decreases the levels of pro-inflammatory cytokines e.g. IL-1β, IL-6, TNF-α, and IFN-γ, while increasing the level of anti-inflammatory cytokines like IL-10. These alterations were predominantly observed after the administration of the CB-2 agonist CBD. Such differential cytokine changes were not observed using THC and other synthetic cannabinoids, which have less effect on this receptor (69, 70).

Based on the data above, investigations have suggested the use of CB-2 agonists as therapeutic medicines to reduce the cytokine storm, for example in case of COVID-19 (71, 72).

1.4.1.1. Cannabinoid effects in endotoxemic conditions

Besides its anti-inflammatory effect, CB-2 receptor activation has been demonstrated to have beneficial vascular effects. CB-2 activation following low-dose, short-term (2 h) LPS treatment has been shown to reduce leukocyte adhesion and to prevent endotoxemic vascular injury in microcapillaries (73). The anti-inflammatory effect of the CB-2 receptor is demonstrated by the observation that wild-type animals exhibited significantly lower cytokine and interleukin levels compared to CB-2 receptor-deficient knock-out animals after 1mg/kg LPS administration. Among other findings, they produced lower levels of Monocyte chemoattractant protein-1 (CCL2), matrix metalloproteinase-9 (MMP-9) and interleukin 6 (IL-6) (74). The beneficial effects mentioned above are contingent upon the immunomodulatory effect of the CB-2 receptor, which reduces the ratio of proinflammatory cytokines and interleukins (75, 76).

THC significantly increased the levels of anti-inflammatory cytokines (e.g.: IL-10) and decreased levels of pro-inflammatory cytokine IL-6 and CCL2. In a study using male and female C57BL/6 mice, animals received 1 mg/kg LPS and 5 mg/kg THC simultaneously. The results confirmed earlier findings: IL-10 levels rises significantly, while proinflammatory cytokines and chemokines gradually decreased over time. The IL-6/IL-10 ratio was significantly reduced (77).

All these results suggest that phytocannabinoids may have a beneficial effect on reducing inflammatory processes under endotoxemic conditions.

1.5. Cannabinoids and the reproductive hormone system

The ECS plays a putative role in the function of the reproductive system. Several studies have demonstrated that in healthy males and females, the administration of exocannabinoids has significantly impaired fertility. In human males, a decrease in sperm count has been observed, while females have experienced cycle length changes and anovulation (78, 79).

Maintaining a delicate balance between endocannabinoid production and degradation, as well as regulating cannabinoid receptor activity, is crucial for optimal reproductive tract and hypothalamic-pituitary-ovarian (HPO) axis function (80, 81).

This is due to the effect of the endocannabinoid system on the hypothalamo - pituitary - gonadal (HPG) axis in the case of females hypothalamo - pituitary - ovarian (HPO) axis. Elevated levels of plasma endocannabinoid or exocannabinoids have been shown to

inhibit gonadotropin releasing hormone (GnRH) release affecting HPO axis function. In chronic exocannabinoid users, reduced luteinizing hormone (LH) surge and follicle stimulating hormone (FSH) levels have been observed (78, 82). In case of males the HPG axis can also be modulated by the ECS. Moreover, CB-1 and CB-2 receptors are present on spermatozoa, and studies indicate that they can influence both the enhancement and reduction of sperm motility (83).

In this context, the most common female endocrine disorder, polycystic ovarian syndrome (PCOS), has also been investigated for endocannabinoid system function. In PCOS patients, plasma AEA levels were significantly increased compared with healthy individuals; and FAAH enzyme and AEA ratios in the ovaries were also altered (Figure 2) (82, 84, 85).

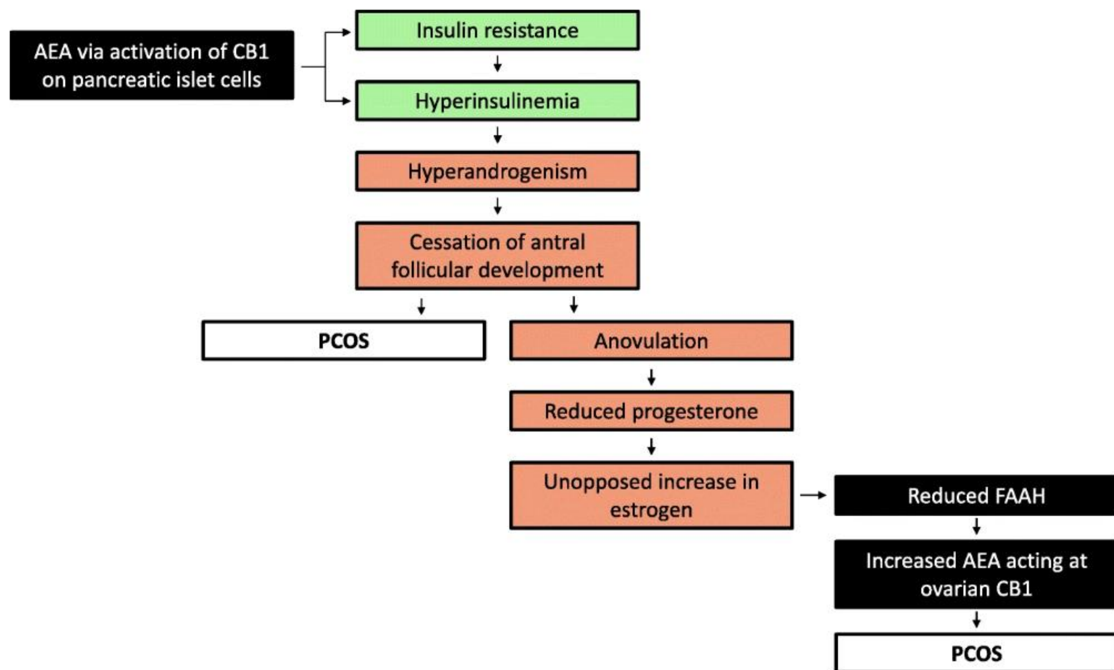

Figure 2. The role of the ECS in the development of PCOS. The primary pathogenic variables associated with PCOS and the impact of certain ECS components are shown. Black boxes represent components of the ECS; green boxes represent metabolic components; orange boxes signify endocrine/reproductive components (82). (CC–BY 4.0)

Abbreviations: AEA – anandamide; CB–1 – Cannabinoid receptor 1; PCOS – polycystic ovarian syndrome; FAAH – fatty acid amide hydrolase;

However, it is important to note that the link between PCOS and endocannabinoids can also be approached from the perspective of metabolic energy homeostasis. It is well

established that the endocannabinoid system plays a key role in regulating the energy balance of the body, which is particularly important in patients with the typically obese, insulin-resistant PCOS phenotype (86).

It has been demonstrated that both the cyclical alternation of endocannabinoid-producing enzymes, and the interaction of female hormones with endocannabinoids are involved in the effect of endocannabinoids on reproductive functions. For example, AEA levels have been found to decrease in proportion to the increase in progesterone levels, which might be due to the effect of progesterone on the enzyme FAAH (87). Furthermore, cannabinoid receptors and 2-AG play a significant role in regulating estrogen levels through the agonism of estrogen receptor beta (ER-β), thereby indirectly influencing the estrogen-GnRH axis. Studies indicate that the agonism of the ER-β receptor significantly reduces the estrogen level, but if we use ER-β antagonists they can elevate the pulsatile secretion of estradiol. This phenomenon shows that cannabinoids can play an important role not only in the energy but also in the reproductive household (88). The expression of the CB-1, CB-2, PPARγ and other receptors in the ovary also varies in the different developmental stages of the follicles (89, 90).

Sexual endocrine disturbance frequently emerges among cannabis (marijuana) and synthetic cannabinoid users, causing cycle abnormalities and infertility (81, 91). The main component of marijuana, THC, blocks the release of GnRH from the hypothalamus, thus inhibiting the production of LH by the adenohypophysis (81, 91, 92). Women who use marijuana may experience a disrupted menstrual cycle, a reduced number of oocytes harvested during in vitro fertilization, and an increased risk of premature delivery or even pregnancy loss (93). The endocannabinoid system (ECS) is believed to play a role in regulating the endometrial cycle. While several components of the ECS, such as CB-1 receptor, CB-2 receptor, and FAAH, have been found to be highly expressed in the female reproductive tract, their function has only been partially explored (94).

An interaction exists between the endocannabinoid system and female reproductive hormones. Specifically, 17β-estradiol (E2) can modulate CB-1 receptor expression and the degradation processes of AEA (95). Additionally, the endocannabinoid system plays a role in controlling ovarian follicle maturation because the female reproductive tract is fully involved with CB-1 and CB-2 receptors. Investigations have shown that AEA levels

vary in a similar way to the female sex cycle, and through this it is able to regulate follicle maturation via the follicle CB-2 receptors (96).

Previous studies have demonstrated that estrogen receptor alpha (ER-α) plays a crucial role in mediating the vasorelaxant effects of estrogen (97). It is important to note that the ER-β activation not only plays a role in estrogen homeostasis, but also has important effects in the regulation of energy homeostasis in addition to sex hormone balance, such as the development of satiety (98) - through these effects it also has direct and indirect cardiovascular impact (99-101).

1.6. Animal models

The most common animal model of the endocannabinoid system is the zebrafish (danio rerio). This is based on the similarity of the endocannabinoid system between zebrafish and humans compared to other animals (rodent models have limitations). Studies of the effects of endocannabinoids on humans are limited in terms of the zebrafish anatomy, but they have proven to be an excellent animal model for studying the effects of endocannabinoids on the signal transduction pathways - given the good genetic variability of zebrafish (knock out and knock in models can be generated). It is also a proven model for studying the dose-dependent toxicity of exocannabinoids. To a limited extent, however, behavioral effects of cannabinoids are also studied in zebrafish, for example to investigate the modulatory effects of cannabinoids on epilepsy (102, 103).

Considering the use of cannabinoids as recreational drugs, the effects of cannabinoids on psychological state and on the body's reward system play an important role in animal studies. Squirrel monkeys have been found to be the most suitable for these experiments. They have been used in both addiction and other self-reward experiments. Similar effects in rodent models have also been carried out using minipump methods (104).

Despite the proven use of zebrafish in cannabinoid systems, rodent models are still the most common in this research area (105, 106).

In animal models of the interaction between cannabinoids and the cardiovascular system, rat and mouse models are the most widely used (107).

There can be minor differences in the presence of receptors, and enzymes of the cannabinoid system between humans and rodents. For example, Cavuoto and colleagues used RT–PCR (real-time polymerase chain reaction) to analyze CB–1, CB–2 receptors,

and FAAH in human and Wistar rat skeletal muscle, finding that while the receptors exhibit slight variations, the abundance of FAAH is nearly equivalent across the species (108). Another team also showed that FAAH and CB–2 receptors are found in mice and rats as well as in human tissues (in this case in bladders) (109). A further study showed a 81% amino acid homology between rodent and human CB–2 receptor (110). Thus, rodent models can be a good alternative to investigate the endocannabinoid system.
Note that other animal models are also used, including dogs and even invertebrates (111-113). To summarize the most potential animal models in the cannabinoid system we have collected them into Table 1.

Table 1. Animal models summarizing table based on: (102, 113, 114)

Model Category	**Species**	**Advantages**	**Main Applications**
Rodent Models	Mice	Genetic manipulation, well-characterized ECS pathways	Receptor function, knockout studies
	Rats	Behavioral studies, pharmacological testing	Drug screening, behavioral assays
Zebrafish	Danio rerio	Transparent embryos, rapid development	Developmental ECS studies, neurogenesis
Non-Human Primates	Monkeys	Closest to human ECS, comparable brain anatomy	Cognition, emotion, social behavior PET imaging
Companion Animals & Livestock	Dogs	Veterinary relevance, translational potential	Pain management, epilepsy treatment
	Cats	Neurological studies, ECS in pain and mood	Mood disorders, neurodegeneration
	Sheep	Endocrine and reproductive ECS studies	Reproductive health, hormone regulation
	Pigs	Digestive and metabolic ECS research	Obesity, metabolic syndrome
Invertebrate Models	Hydra	Simple nervous systems, evolutionary insights	Evolutionary conservation of ECS

	Sea Urchins	Developmental biology, ECS in regeneration	Regeneration and repair mechanisms
	Leeches	Neurophysiology, ECS in reflex modulation	Motor control, ECS in reflexes
	Nematodes	Genetic simplicity, ECS in aging and stress	Longevity, stress response

2. OBJECTIVES

Delta-9-tetrahydrocannabinol effect on the vasorelaxation and oxidative-nitrative stress in endotoxemia

Endotoxin shock and endotoxemia leads to altered cardiovascular function that plays an important role in their mortality. Deteriorated endothelial function is one of the first signs of cardiovascular dysfunction. Increased pro-inflammatory processes and consequently increased oxidative-nitrative stress are strongly connected to endothelial dysfunction. The acute anti-inflammatory properties of THC may alleviate the progression of endotoxin shock and promote better cardiovascular function. In the present study our aim was to investigate the possible beneficial effect of THC treatment on the endothelial function in a rat model of endotoxemia and its role in alterations of vascular relaxation mechanisms, and oxidative-nitrative stress.

Effect of CB-1 receptor deficiency on vascular tissue in female aorta

The endocannabinoid system is well known of its sex differences, in which the interaction between the endocannabinoid system and the reproductive endocrine system may play a major role. Our research group observed and increased conjugated estradiol plasma concentration in CB-1 knockout female mice compared to their wild type littermates. These animals also showed increased acetylcholine and estrogen induced relaxation of their isolated aortas (115). In our second study our aim was to identify changes in the vascular structure as well as the possibly altered presence of enzymes and receptors of vasoactive substances and oxidative-nitrative stress markers that may play role the improved endothelial function of these animals.

3. METHODS

3.1 Delta-9-tetrahydrocannabinol effect on the vasorelaxation and oxidative-nitrative stress in endotoxemia

3.1.1. Animals

32 age-matched male Sprague-Dawley rats, with an average weight of 300 g (around 12 weeks old), were divided into three groups. The animals were observed for 24 hours after receiving the following treatments.: the control (n=12) group was treated with intravenous saline injection (as a vehicle for the lipopolysaccharide (LPS) in the LPS treated group) and intraperitoneal solvent (alcohol – saline mix as a vehicle for the next group's THC treatment). The THC treated group (n=8) got a single dose of 10mg/kg intraperitoneal THC (Sigma-Aldrich, St.Louis, MO, USA) solubilized in ethanol:saline mix: 1:2.5 ratio. 10 minutes later the animals were administered 5 mg/kg LPS (from Escherichia coli, Sigma Aldrich, St. Louis, MO, USA) intravenously, while another 12 animals were treated only with intravenous LPS.

Table 2.: In vivo treatment layout

Abbreviations: LPS- lipopolysaccharide; THC - delta 9 tetrahydrocannabinol

Groups	**CONTROL (N=)**	**LPS**	**LPS+THC**
LPS 5 mg/kg iv.	**0**	+	+
THC 10 mg/kg ip.	**0**	**0**	+

After LPS administration, the animals remained in their usual environment for 24 hours under surveillance because of the perception of suffering (from endotoxemia induced hyperalgesia, fatigue, and anxiety) caused by the LPS treatment. During this time, we did not use anesthetics because it would have interfered with the results of the experiment. Measurements and collection of tissue specimens took place 24 hours after LPS treatment under anesthesia with i.p. injections of 60 mg/kg thiopentone sodium (Nembutal, Phylaxia-Sanofi, Hungary). In deep anesthesia the chest of the animals were opened, blood samples from the heart and after saline perfundation, thoracic aorta sections were collected for further analysis.

All investigations conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, Revised 1985) and all procedures were approved by the Semmelweis University Committee on the Ethical Use of Experimental Animals (590/99 Rh).

3.1.2. Myography of isolated aortic rings

The thoracic aorta was cleared from periadventitial fat and cut into 3–4 mm width rings, mounted in organ baths filled with warmed (37 ˚C) and oxygenated (95% O_2, 5% CO_2 – Carbogen Lindegas®) Krebs' solution ($CaCl_2$ 1.6 mM; $MgSO_4$ 1.17 mM; NaCl 130 mM; $NaHCO_3$ 14.9 mM; KCl 4.7 mM; KH_2PO_4 1.18 mM; Glucose 11 mM). Isometric tension was measured with isometric transducers (10 cm^3 capacity, vertical training organ bath system, Experimetria Ltd. Budapest, Hungary.) A tension of 1.5 gram was applied and the rings were equilibrated for 60 minutes, followed by an epinephrine dose-response curve (10^{-10}–$3*10^{-6}$ M) and, after a 30- to 60-minute-long washout period, the rings were precontracted with epinephrine (10^{-7} M) and concentration-dependent relaxation to acetylcholine (Ach, 10^{-9} to $3*10^{-4}$ M) was measured.

3.1.3. Measurement of systemic oxidative stress

Serum was isolated for malondialdehyde detection. The blood samples were stored at -80˚C until the time of analysis. Samples were homogenized with 0.5 ml of 1.15% KCl solution and centrifuged at 5000 rpm for approximately 30 min until the supernatant was completely clear. On a standard microplate the following solutions were added to the wells: sodium dodecyl sulfate (8.1%), acetic acid (20%), water, the supernatant, thiobarbituric acid (0.8%) and incubated at 95˚C for one hour. The concentration of thiobarbituric acid reactive products was measured by photometry at 532 nm (PowerWave XS, BioTek Instruments, CA, USA.)

3.1.4. Immunohistochemistry

Immunohistochemistry was performed on paraformaldehyde (PFA) fixed paraffin-embedded 7µm thick tissue sections of the thoracic aorta against poly(ADP–ribose) polymers (PAR), cannabinoid receptor 1 and 2 (CB–1R and CB–2R); COX–2, endothelial nitric oxide synthase (eNOS) 4–hydroxynonenal (HNE), cyclic guanosine–

monophosphate (cGMP) and 3'–nitrotyrosine (NT). After deparaffinization, antigens were retrieved by heating the slides in citrate buffer (pH = 3 for PAR, CB–2R or pH = 6 for CB–1R, COX–2, eNOS and HNE; for cGMP and NT, we did not apply antigen retrieval). Endogenous peroxidase activity was blocked with 3% H_2O_2 in distilled H_2O. Nonspecific labeling was blocked using 2.5% normal horse serum (Vector Biolabs, Burlingame, CA, USA). After blockage overnight application of the following primary antibodies at 4˚C were taken:

(monoclonal mouse anti-eNOS 1:50, Abcam, Cambridge, UK, RRID:AB_1310183; polyclonal rabbit anti-COX-2 1:200, Abcam, Cambridge, UK, RRID:AB_2085144; polyclonal rabbit anti-cGMP 1: 500, Merck Millipore, Burlington, MA; USA, RRID:AB_568797, polyclonal rabbit anti-NT 1: 500, Merck Millipore, Burlington, MA; USA. RRID:AB_310089, polyclonal rabbit anti-HNE 1:200, Abcam, Cambridge, UK, RRID:AB_722490; monoclonal mouse anti-PAR 1:500 Abcam, Cambridge, UK, RRID:AB_301239; polyclonal rabbit anti-CB−1R 1:200, Cayman Chemical, Ann Arbor Michigan U.S.A. RRID:AB_327840; polyclonal rabbit anti-CB−2R 1:150, Fabgennix, Thermo Fisher Scientific, Waltham, MA, U.S.A. Cat. number: CB−2R-201AP) (See these antibodies in Table 3).

Table 3: Primary antibodies used in immunohistochemistry in this study

ANTIBODY	USED CONCENTRATION	HOST ANIMAL	VENDOR	RRID
eNOS	1:50	mouse	Abcam	1310183
COX-2	1:200	rabbit	Abcam	2085144
cGMP	1:500	rabbit	Merck Millipore	568797
NT	1:500	rabbit	Merck Millipore	310089
HNE	1:200	rabbit	Abcam	722490
PAR	1:500	mouse	Abcam	301239
CB-1	1:200	rabbit	Cayman chemical	327840

CB-2	1:150	rabbit	Fabgennix	Cat. number: CB2R-201AP

On the next day, horseradish-peroxidase- (HRP-) linked anti-mouse (PAR, eNOS) or anti-rabbit (NT, HNE, CB-1R, CB-2R, cGMP, COX-2) horse antibodies (Vector Biolabs, Burlingame, CA, USA.) provided secondary labeling, which was visualized by brown-colored diamino-benzidine (DAB, Vector Biolabs, Burlingame, CA, USA.). For counterstaining, blue-colored hematoxylin (Vector Biolabs, Burlingame, CA, USA.) was utilized. Microscopic imaging of tissue sections was performed with a Nikon Eclipse Ni-U microscope with DS-Ri2 camera (Nikon, Minato, Tokyo. Japan). The positively stained area (brown coloration) over the whole tissue area (area%) of the endothelium (eNOS and COX-2) and of the media (CB−1R, HNE, NT, cGMP); as well as the positively stained nuclear area over the whole nuclear area in the media (nuclear area%, PAR) were estimated by ImageJ software (NIH, Bethesda, MA, USA).

3.1.5. Statistical analysis

Values were expressed as mean ± Standard Error of Mean (mean ± SEM). Statistical significance between groups was determined by repeated measure two-way analysis of variance (ANOVA) with Bonferroni's multiple comparison and non linear regression test with pairwise comparisons to evaluate the bottom of the data sets (aortic rings), one-way ANOVA with Tukey's multiple comparison (malondialdehyde assay), or Kruskal-Wallis test with Dunn's post hoc test (in case of non-gaussian data distribution). A significance threshold was set at $p < 0.05$, Analysis was performed by GraphPad PRISM 9.5.0. (San Diego, CA, USA).
All data about this topic have been mentioned originally in our research article (116).

3.2. Effect of CB-1 receptor deficiency on vascular response in female aorta

3.2.1. Animals and chemicals

Female homozygous 4–6 months old (20–23 g) CB–1R–knockout mice (CB–1R KO, CB–1R$^{-/-}$, n = 25) originally from Andreas Zimmer (117) and their wild–type counterparts (CB–1R$^{+/+}$; n = 35) were used. Animals were anesthetized with pentobarbital (Euthasol,

ASTPharma, Oudewater, Netherlands 50 mg/kg) injected peritoneally, and an additional dose of Euthasol (5–10 mg/kg) was given if needed. After anesthesia, animals were perfused with saline via the left ventricle and the abdominal aorta was isolated. Upper segments of the abdominal aorta were isolated for immunohistochemistry.

The investigations were conducted in accordance with the instructions of the Guide for the Care and Use of Laboratory Animals (NIH 8th Edition 2011), Institutional and National guidelines for animal care and were approved by the Animal Care Committee of the Semmelweis University, Budapest and by the Hungarian authorities (No. PE/EA/1428-7/2018).

3.2.2. Histological and immunohistochemical stainings

Paraformaldehyde (PFA)-fixed, paraffin-embedded abdominal aortic sections, in 7 µm thickness, were cut. Hematoxylin eosin staining (HE) was used for topographical analysis, e.g., intima–media ratio, and to examine morphological changes. Incubation with hematoxylin (Hematoxylin modified to Gill II, Sigma-Aldrich, St. Louis, MO, USA); followed by ethanol washing, incubation with eosin (Eosin Y, Merck Millipore, Burlington, USA) was performed. Elastic fibers were stained with resorcin fuchsin (Electronmicroscopy Sciences, Hatfield, PA, USA).

Sections were immunohistochemically labelled against α – smooth muscle actin (α–SMA), eNOS, COX–2, TP receptor (TP), thromboxane A synthase (TBXAS), estrogen receptor α and β (ER–α; ER–β) and NT. After deparaffinization, antigen retrieval was performed by heating the slides in citrate buffer (pH = 6) in case of α–SMA, eNOS, COX–2, TBXAS and ER–β. Proteins were digested with Proteinase K (1 mg/mL in phosphate buffer) (Merck Millipore, Burlington, MA, USA) for ER–α and NT stainings. Endogenous peroxidase activity was blocked with 3% H_2O_2. To prevent nonspecific labeling of the secondary antibody, we used a 2.5% normal horse serum (NHS) blocking solution (Vector Biolabs, Burlingame, CA, USA). Primary antibodies were applied overnight at 4°C were as follows: α–SMA mouse monoclonal antibody 1:10,000 (Abcam, Cambridge, UK RRID:AB_262054); eNOS mouse monoclonal antibody 1:50 (Abcam, Cambridge, UK RRID:AB_1310183; COX–2 rabbit polyclonal antibody 1:200 (Abcam, Cambridge, UK); TBXAS mouse monoclonal antibody 1:50 (Invitrogen Waltham, MA, USA RRID:AB_2723709); ER–β rabbit polyclonal antibody 1:500 (Invitrogen Waltham,

MA, USA RRID:AB_325597); TP rabbit polyclonal antibody 1:50 (MyBioSource, San Diego, CA, USA RRID:AB_2811271); ER−α rabbit polyclonal antibody 1:80 (Merck Millipore, Burlington, MA, USA RRID:AB_310305); NT rabbit polyclonal antibody 1:250 (Merck Millipore, Burlington, MA, USA RRID:AB_310089) (See the antibodies in Table 4).

Table 4: Primary antibodies used in immunohistochemistry in this study

ANTIBODY	USED CONCENTRATION	HOST ANIMAL	VENDOR	RRID
α−SMA	1:10000	mouse	Abcam	262054
eNOS	1:50	mouse	Abcam	1310183
COX-2	1:200	rabbit	Abcam	2085144
TBXAS	1:50	mouse	Invitrogen	2723709
TP	1:50	rabbit	MyBiosource	2811271
ER−α	1:80	rabbit	Merck Millipore	310305
ER−β	1:500	rabbit	Invitrogen	325597
NT	1:500	rabbit	Merck Millipore	310089

For secondary labeling we used horseradish−peroxidase (HRP) linked anti−mouse (in case of α−SMA, eNOS, TBXAS stainings) or anti−rabbit (in case of ER−α , ER−β , COX−2, TP and NT stainings) IgG polyclonal antibodies were used (Vector Biolabs, Burlingame, CA, USA). Visualization was performed by brown colored horse radish peroxidase (HRP) linked 3,3'-diaminobenzidine precipitation (DAB, Vector Biolabs, Burlingame, CA, USA). For counterstaining hematoxylin was applied (Hematoxylin modified to Gill II, Sigma-Aldrich, St. Louis, MO, USA).

After covering, slides were photographed using Nikon eclipse Ni-U microscope with DS-Ri2 camera (Nikon, Minato, Tokyo, Japan). Photos of the eNOS, COX−2 and ER−α slides were taken at 20x magnification, in other cases (including HE and RF stainings) we used 10x magnification. On HE slides the thickness of the aortic wall, and separately the thickness of the intima and media layers, were measured. Non−calibrated optical density (O.D.) on the RF-stained slides characterized the elastic fiber density. On immunohistochemical slides, the brown positivity and the background staining (DAB +

hematoxylin) were separated, staining intensity was determined by non−calibrated optical density of the brown color. In the case of eNOS and COX−2, only the endothelial layer was evaluated, while in the case of ER−α and ER−β both the endothelial and the media layers were evaluated separately. In the remaining cases cases, the staining intensity was investigated in the media layer using the FIJI® software (National Institutes of Health, Bethesda, MA, USA).

3.2.3. Statistical analysis

Results were tested with unpaired t-test. Values were expressed as mean ± standard error of the mean (mean ± SEM), significance threshold was set at $p < 0.05$, Analysis was performed by GraphPad PRISM 9.5.0. (San Diego, CA, USA).
All data about this topic have been mentioned originally in our research article (115).

4. RESULTS

4.1. Delta-9-tetrahydrocannabinol effect on the vasorelaxation and oxidative-nitrative stress in endotoxemia

4.1.1. Vascular functions

Following epinephrine (10^{-7} M) precontraction, applying 10^{-9} to 10^{-5} M concentrations of acetylcholine (Ach) led to arterial relaxation that was much lower in the LPS group than in the control vessels. While the LPS+THC group did not show significant differences compared to the control group above 10^{-7} M Ach concentration, so THC co-treatment was able to prevent the LPS induced endothelial dysfunction (Fig 3 – Table 5).

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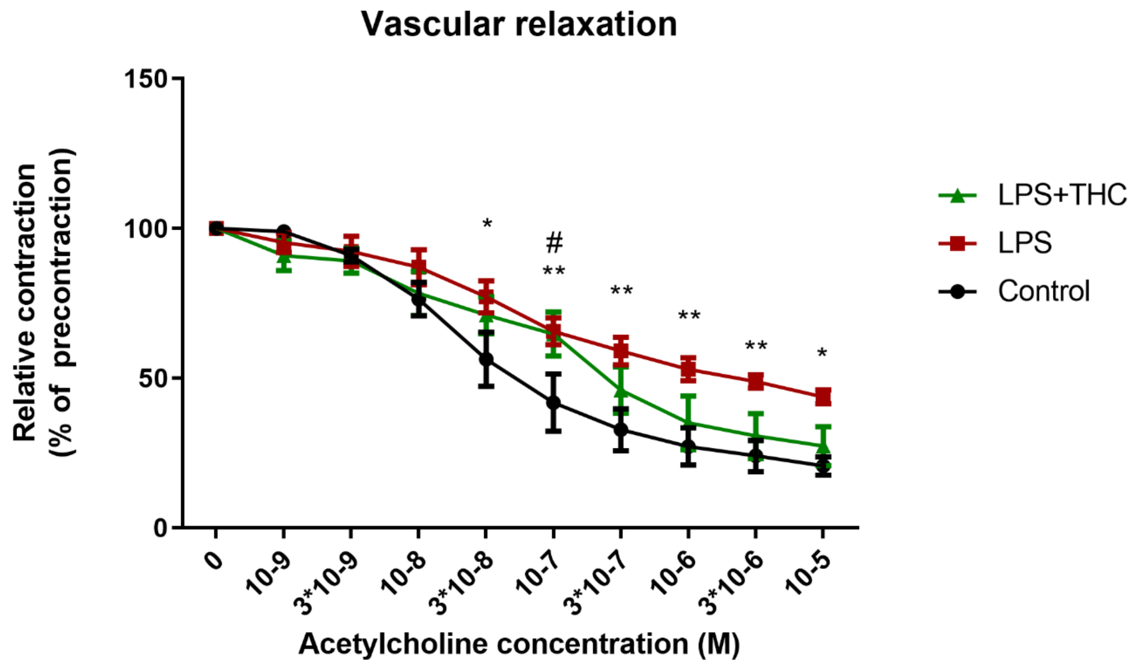

Figure 3. Acetylcholine-induced relaxation of isolated thoracic aorta segments after epinephrine precontraction. LPS treatment (squares) caused a reduced endothelium-dependent acetylcholine-induced relaxation compared to the Control (circles). THC treatment (triangles) prevented the decrement in the endothelium-mediated relaxation. Data are presented as mean ± SEM, n = 4 animal in each group; repeated measures two-way ANOVA, Bonferroni's post hoc test, *p<0.05 Control vs. LPS, **p<0.01 Control vs. LPS, #p<0.05 Control vs. LPS+THC.

For better characterization of the dose-response curves, we also calculated the bottom values of each group, which show significant difference between all experimental groups (Table 5).

Table 5: Bottom values of the groups based on Ach induced relaxation

GROUP	Bottom	Bottom 95% confidence interval
Control	24,35	18,43 - 30,05
LPS	48,18	43,10 - 52,91
LPS+THC	$2.756*10^{-7}$M	19,65 - 37,62

Following pairwise comparisons and p correction for the number of comparisons:

Control vs. LPS: $p<0.003$

Control vs. LPS+THC: $p = 1.1067$

LPS vs. LPS+THC: $p=0.0009$

4.1.2. Vasoactive markers of the aortic wall

LPS had no effect on the density of eNOS, while the density of the LPS+THC group was significantly lower than that of the control group (Fig 4. Panel A, B;). Similar trends can be seen in COX-2 density, where the staining intensity of the LPS+THC group was considerably lower than that of the Control group (Fig 4. Panel C, D). In contrast, the aortic wall of the LPS rats showed a substantial decrease in cyclic guanosine monophosphate (cGMP) staining when compared to the Control group (Fig 4. Panel E, F).

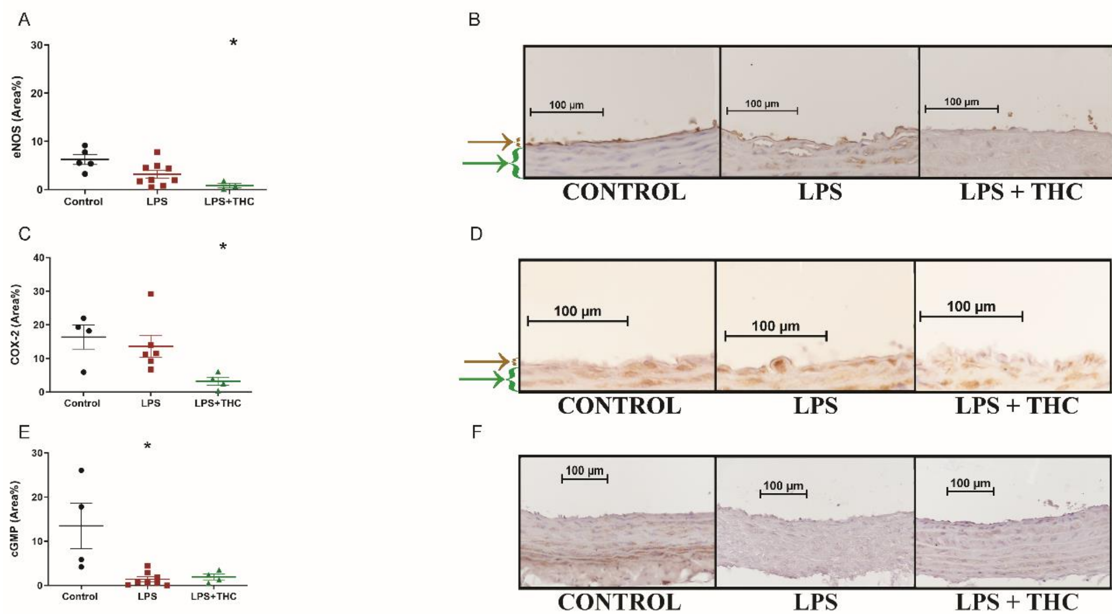

Figure 4. Histological changes of the vasoactive markers in the thoracic aorta

Panel (A) eNOS density of the aorta's endothelial layer. Data shows positive area % of area of the endothelial layer as mean ±SEM (n = 5–9–3) *:p<0.05 Control vs. LPS+THC Panel (B) Representative images of eNOS stained aorta segments focusing on the endothelial layer.

Panel (C) COX–2 density in the aorta segments. Data shows positive area % of the area of the endothelial layer as mean ±SEM; (n = 4–6–4) *:p<0.05 Control vs. LPS+THC.

Panel (D) Representative images of COX-2 labelled aorta segments in the endothelial layer.

Panel (E) cGMP density of the aorta segments. Data shows positive area % of the total aortic tissue area as mean ±SEM; (n = 4–7–4) *:p<0.05 Control vs. LPS.

Panel (F) Representative images of cGMP labelled aorta segments.

Panels B, and D: Brown arrows shows the intima layer, and green arrows shows the media layer of the vessels.

Panels B, D, F: Brown precipitate (3' diaminobenzidine) represents positive staining with blue hematoxylin counterstaining. Photos were taken with 20x magnification in case of eNOS and COX-2 representative photos, cropped to focus to the endothelial layer, while 10x magnification in case of cGMP representative photos focus to the media layer.

In all cases, statistical analyses were performed using Kruskal-Wallis test & Dunn's post-hoc test.

Regarding CB−1R, the specific staining revealed a decrease in density in the LPS treated group compared to the Control group (Fig. 5. Panel A, B). Furthermore, there was a clear downward trend in the density of CB−2R in the LPS group in comparison to the Controls ($p = 0.0548$). In contrast to CB−1R staining, THC-treated rats had CB−2R abundance that was more comparable to Controls (Fig. 5, Panel C, D).

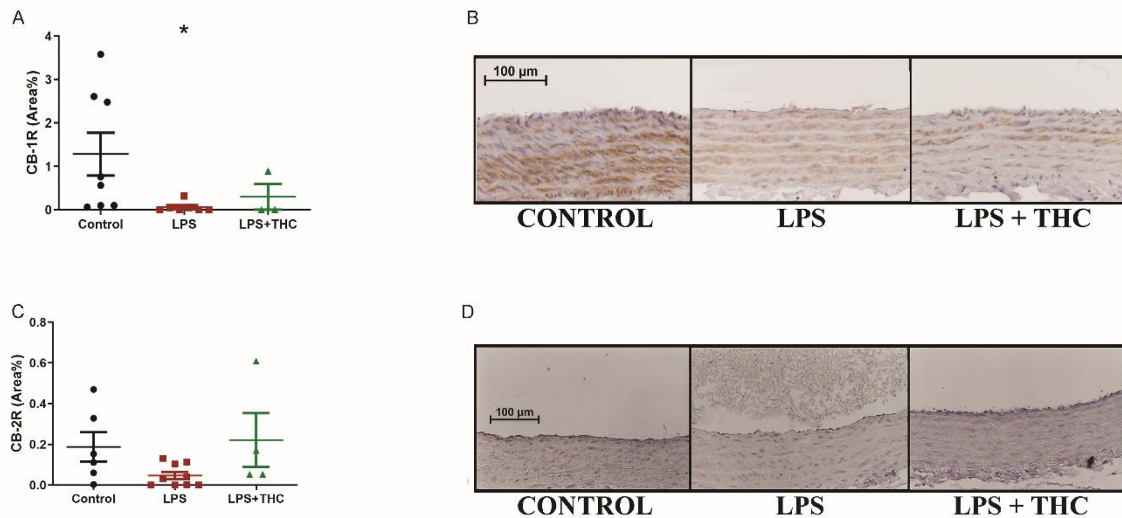

Figure 5. Histological changes of the cannabinoid receptor 1 and 2 intensity in the thoracic aorta

Panel (A) CB−1R density of the aorta segments. Data shows positive area % of the whole aortic tissue area as mean ±SEM; (n = 8–7–3) *:$p<0.05$ Control vs. LPS.

Panel (B) Representative images of CB−1R stained aorta segments

Panel (C) CB−2R density of the aorta segments. Data shows positive area % of the whole aortic tissue areas mean ±SEM; (n = 6–9–4).

Panel (D) Representative images of CB−2R stained aorta segments

Panels B, D - The positivity labeled with 3,3' diaminobenzidine with brown precipitate, and a violet color hematoxylin counterstaining, 10x magnification with image cropped to focus on the media layer.

In all cases, statistical analyses were performed using Kruskal-Wallis test & Dunn's post-hoc test (116).

4.1.3. Oxidative-nitrative stress markers

The malonyl-dialdehyde assay (MDA) was utilized to evaluate systemic oxidative stress. The results showed that the LPS-treated group had a higher level of oxidized circulating byproduct; however, this elevation was not statistically significant compared to the LPS + THC group (Fig 6. Panel A). Tissue oxidative stress was assessed based on the immunohistochemical staining of 4-hydroxy-nonenal (HNE) in aortic samples. Tissues from the LPS group had significantly higher staining intensities in the aortic wall (Fig. 6, Panels B–C). In the aortic wall, an elevation of both NT and PAR was detectable only in the LPS group (Fig 6. Panel D-G).

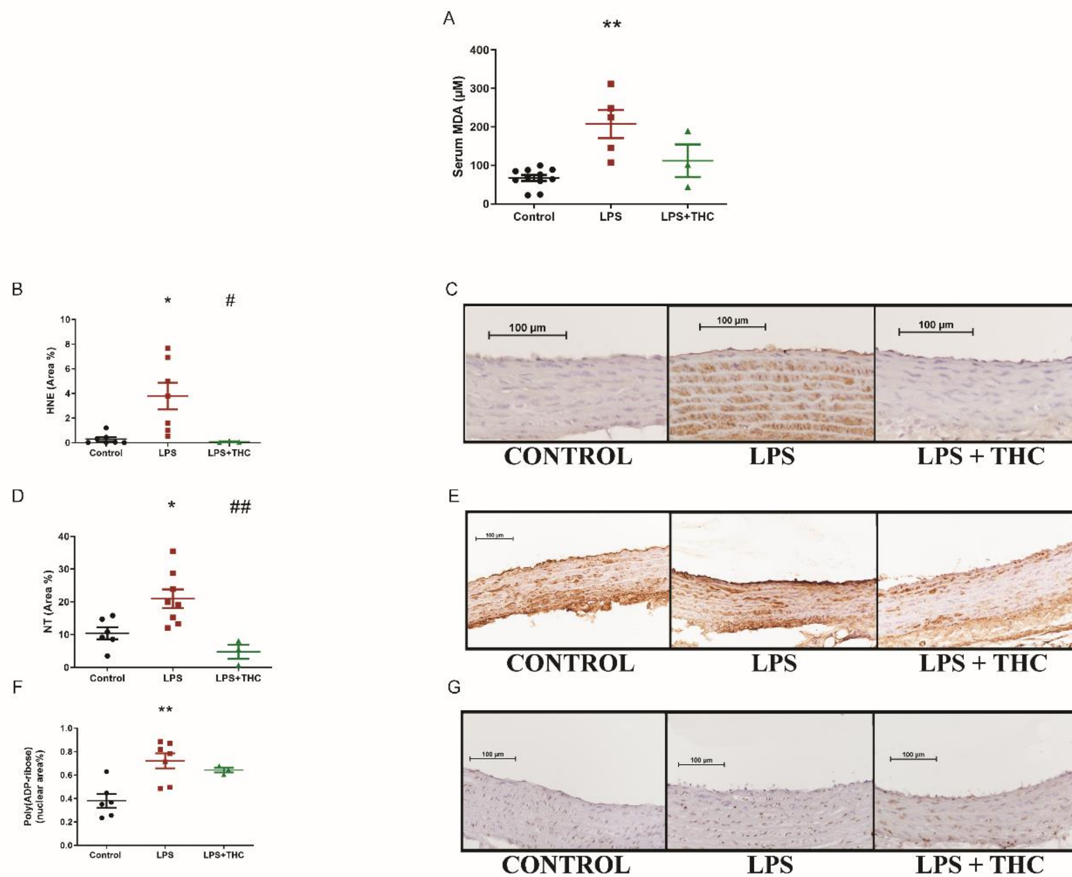

Figure 6. Oxidative- nitrative stress markers

Panel (A) Systemic oxidative stress detected by malonyl-dialdehyde assay (n = 11–5–3) was significantly elevated in endotoxemic rats compared to the controls, but not in THC-treated animals. **p<0.01 vs. Control.

Panel (B) HNE staining positive area%, in the thoracic aortic wall showed a significant elevation in the LPS group compared to the controls, but not in LPS+THC animals. *p<0.05 vs. Control, #p<0.05 vs. LPS, (n = 7–7–3).

Panel (C) Representative images of the aortae stained against HNE.

Panel (D) Assessing nitrative stress. NT positive area%, in the thoracic aortic wall showed a significant elevation in LPS group, but not in LPS+THC treated animals. *p<0.05 vs. Control, ##p<0.01 vs. LPS, (n = 6–8–3)

Panel (E) Representative images of the aortae stained against NT.

Panel (F) DNA damage assessed by Poly(ADP-ribose) polymer nuclear density (PAR, positive nuclear area%) in the thoracic aortic wall showed a significant elevation in LPS group, but not in LPS+THC animals. **p<0.01vs. Control. (n = 6–7–3)

Panel (G) Representative images of the aortae stained against PAR.

Panels C, E and G: Brown precipitate (3,3' diaminobenzidine) represents positive staining with violet hematoxylin counterstaining. Photos were taken with 10x magnification – in the case of HNE staining photos were cropped to focus on the media layer.

In all cases, statistical analysis was executed with Kruskal-Wallis test & Dunn's post-hoc test (116).

We summarized our results in Table 6.

Table 6: Summarizing table of the results

	LPS	LPS+THC
Ach induced relaxation	↓↓	↓
endothelial eNOS	-	↓
endothelial COX-2	-	↓
vessel cGMP	↓	-
vessel CB-1R	↓	-
vessel CB-2R	-	-
Serum MDA	↑	-
vessel HNE	↑	-
vessel NT	↑	-
vessel PAR	↑	-

4.2. Effect of CB-1 receptor deficiency on vascular response in female aorta

4.2.1. Vascular remodeling

The intima–media ratio shows a significant decrease in the CB−1R KO group in comparison to the WT group as a result of the media layer thickening (Fig 7 A, C). The overall wall thickness was increased as well (Fig 7 B–C). No significant difference in elastic density, as assessed by RF staining, was observed between the two strains (Fig 7 D, E). Stainings of α–smooth muscle actin (α–SMA) did not reveal any noticeable differences between the CB−1R KO and the WT groups (Fig 7 F, G).

Based on the intima media ratio, and the increased wall thickness we present also the intima and media layer thicknesses separately where the thickness of the media layers significantly elevated in the CB−1R KO group, but the intima layers shows no significant difference (Fig 8 A, B).

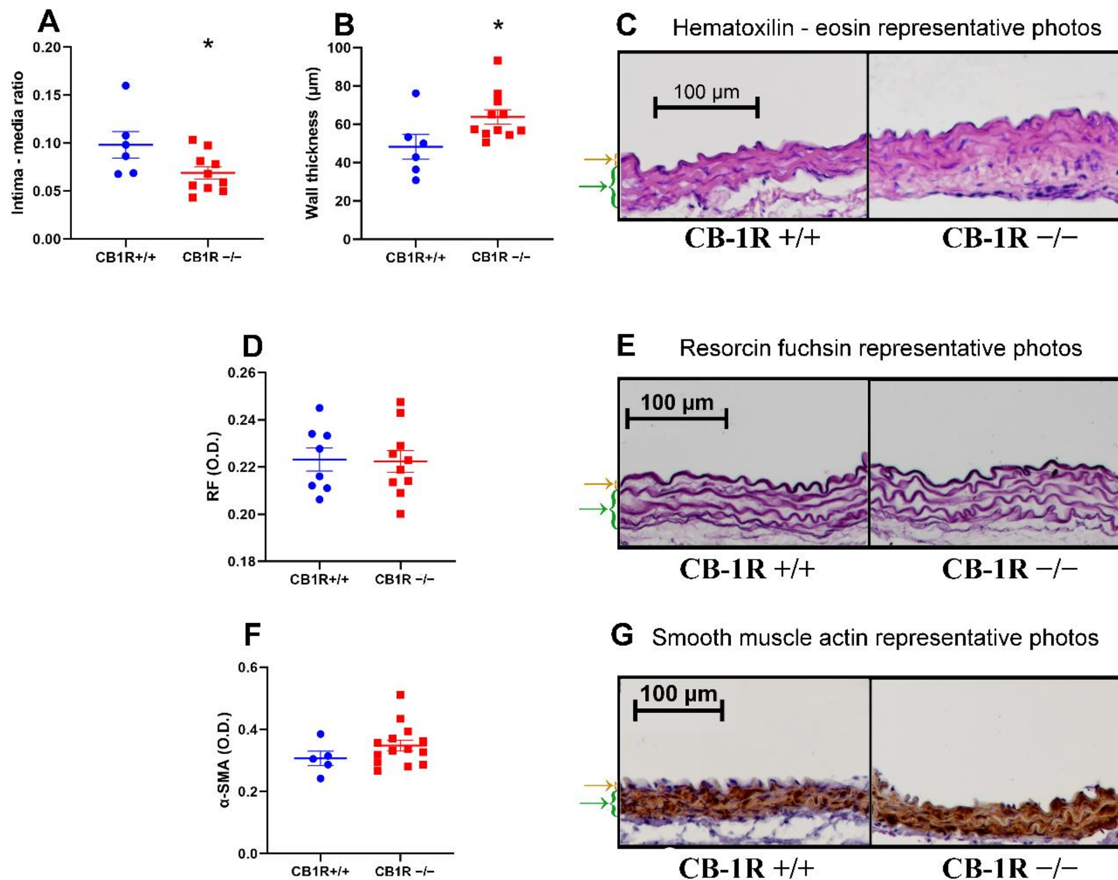

Figure 7. Morphological changes of the abdominal aorta wall

Panel (A). Intima–media ratio of the aorta walls as mean ± SEM, n = 6 (CB−1R+/+) n = 10 (CB−1R-/-) *: p < 0.05 CB−1R+/+ vs. CB−1R-/- group.

Panel (B). Aorta wall thickness in micrometer as mean ± SEM n = 6 (CB−1R+/+) n = 11 (CB−1R-/-) *: p < 0.05 CB−1R+/+ vs. CB−1R-/- group.

Panel (C). Representative images of the hematoxylin-eosin staining photographed at 10x magnification.

Panel (D). Optical density of elastic fiber on resorcin fuchsin stained sections. n = 7 (CB−1R+/+), n = 10 (CB−1R-/-).

Panel (E). Representative images of the resorcin fuchsin stained sections photographed at 10x magnification.

Panel (F). Optical density of α−SMA-stained sections. n = 5 (CB−1R+/+) n = 14 (CB−1R-/-)

Panel (G). Representative images of α−SMA-stained aorta segments, visualization with diaminobenzidine (DAB) on hematoxylin counterstaining, photographed at 10x magnification.

Panel C, E, G: Brown arrows shows the intima layer, and green arrows shows the media layer of the vessels.

Statistical analysis was performed by unpaired t-test. Data are presented as non−calibrated optical density with mean ± SEM.

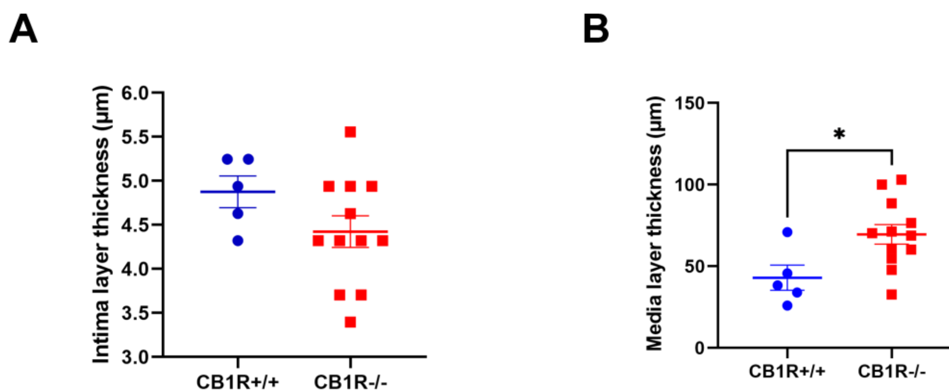

Figure 8. Intima and media layer thickness of the abdominal aorta wall

Panel (A). Intima layer thickness of the aorta walls in micrometer as mean ± SEM, n = 5 (CB−1R+/+) n = 12 (CB−1R-/-) No significant difference between the groups.

Panel (B). Intima layer thickness of the aorta walls in micrometer as mean ± SEM n = 5 (CB−1R+/+) n = 12 (CB−1R-/-) *: $p < 0.05$ CB−1R+/+ vs. CB−1R-/- group.

4.2.2. Vasoactive markers — molecular contributors of vascular functions

The CB−1R KO group demonstrated a significant elevation in eNOS protein density in the aortic wall, as compared to the WT group (Fig 9 A, B). The study observed a significant decrease in COX−2 in the endothelium in the receptor deficient animals (n = 8 and 12 for the WT and CB−1R KO groups, respectively) (Fig 9 C, D). The study found no significant differences in the density of the TP receptor density between the WT group and the CB−1R KO group (Fig 9 E, F). Unlike TP density, we observed a trend for the level of TXBAS to drop in CB−1R KO aortas compared to wild−type vessels. However, this decrease did not achieve statistical significance (p=0.06, unpaired t test) (Fig 10).

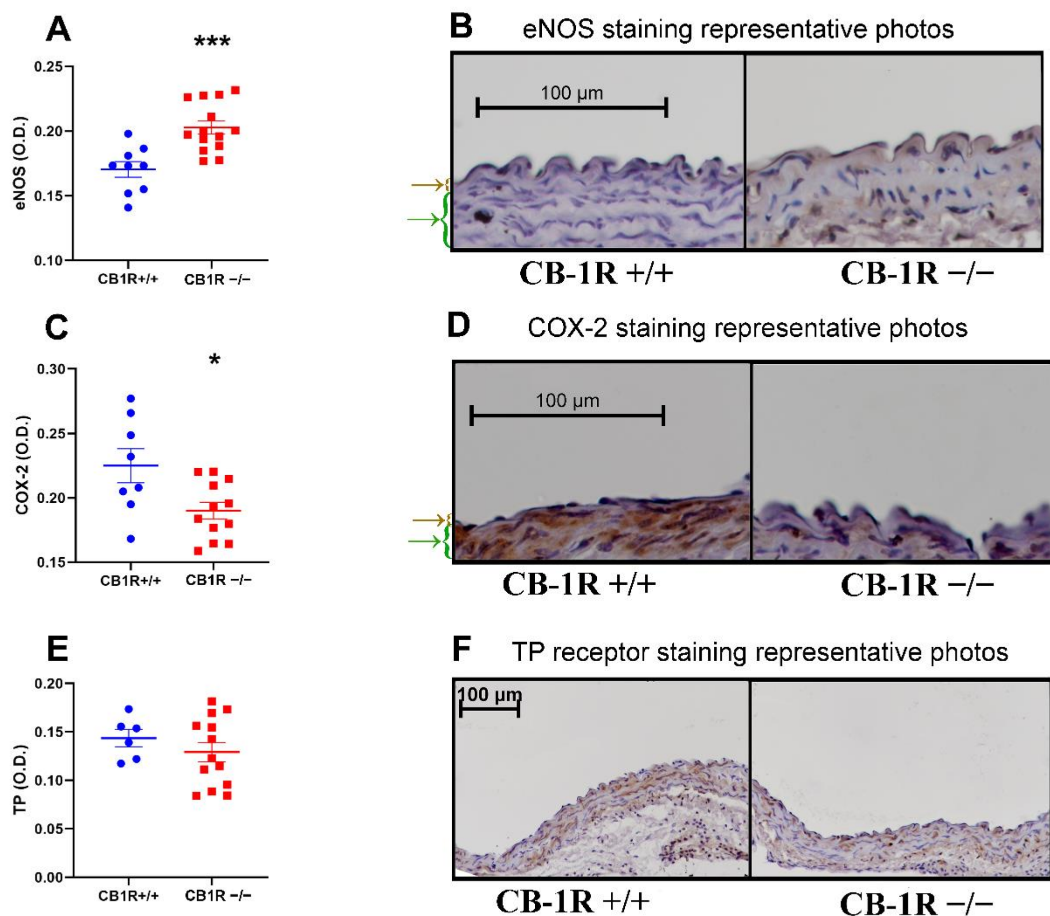

Figure 9. Vasoactive markers of the abdominal aorta wall

Panel (A). Results of eNOS immunostained sections: The eNOS density of the CB−1R-/- group was significantly increased compared to the wild-type animals. $n = 9$ (CB−1R+/+) $n = 14$ (CB−1R-/-). ***: $p < 0.001$ CB−1R+/+ vs CB−1R-/- group.

Panel (B). Representative images of eNOS immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed at 20× magnification. Evaluation performed from the values of the endothelial layer

Panel (C). Results of COX−2 immunostained sections: Antagonistic to the eNOS results COX−2 density. significantly decrease in the KO group. $n = 8$ (CB−1R+/+), $n = 12$ (CB−1R-/-). *: $p < 0.05$ CB−1R+/+ vs. CB−1R-/- group.

Panel (D). Representative images of COX−2 immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed by 20× magnification. Evaluation performed from the values of the endothelial layer.

Panel (E). Results of TP receptor immunostained sections. $n = 6$ (CB−1R+/+), $n = 13$ (CB−1R-/-).

Panel (F). Representative photos of TP receptor immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed by 10× magnification. Evaluation was performed from the values of the media layer.

Panel B, and D: Brown arrows shows the intima layer, and green arrows shows the media layer of the vessels.

Statistical analysis was performed with unpaired *t*-test. Data are shown as non−calibrated optical density with mean ± SEM.

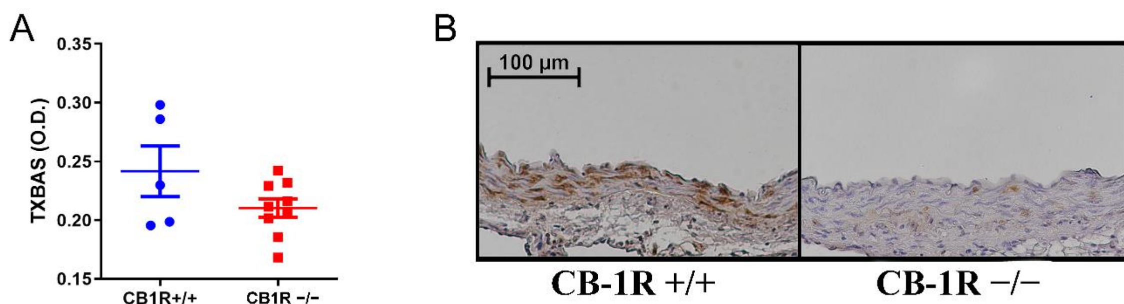

Figure 10. Thromboxane synthase density of the abdominal aorta wall

Panel A. Results of immunohistochemical staining of thromboxane synthase (TXBAS) on aortas of wild–type (CB−1R+/+, n=5) and CB−1R KO (CB−1R-/-, n=9) mice. We have not detected any significant difference.

Panel B. Representative images of TXBAS immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed at 20x magnification. Evaluation was performed from the values of the media layer. Statistical analysis was performed with unpaired t-test. Data are shown as non-calibrated optical density with mean ± SEM (115).

4.2.3. Estrogen receptor density

No significant differences were observed in the density of ER−α in the endothelium or media layers of the aorta wall between groups (Fig 11 A–C). In regards to ER−β, on the other hand, we discovered that CB−1R KO animals had reduced expression in both the media and endothelial layers (Fig 11 D–F).

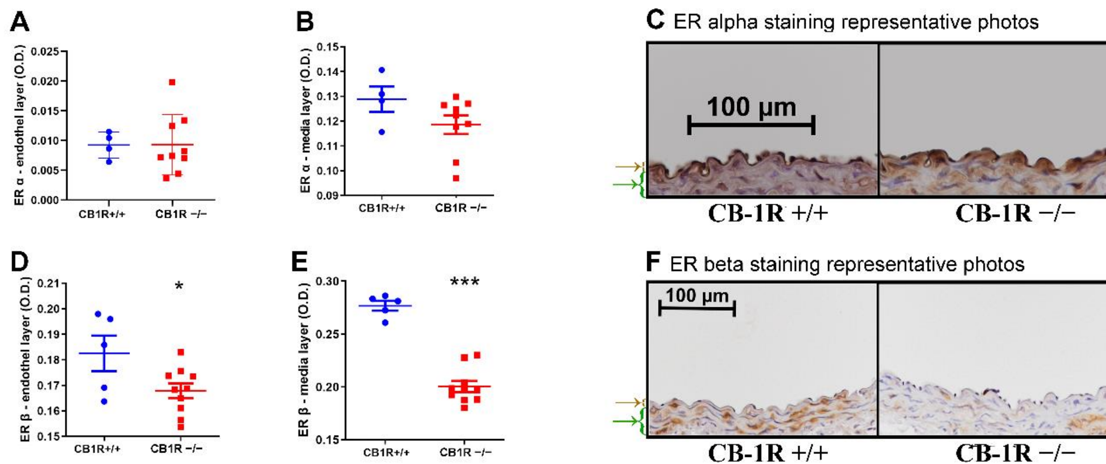

Figure 11. Estrogen receptor α and β density of the abdominal aorta wall (115)

Panel (A). ER−α density in the endothelial layer. *n* = 6 (CB−1R+/+), *n* = 9 (CB−1R-/-). No significant difference between the groups.

Panel (B). ER−α density in the media layer. n = 4 (CB−1R+/+) n = 9 (CB−1R-/-). no significant difference between the groups.

Panel (C). Representative images of ER−α immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed at 20× magnification. Evaluation performed from the values of the endothelial and the media layer.

Panel (D). ER−β density in the endothelial layer. $n = 5$ (CB−1R+/+), $n = 9$ (CB−1R-/-). We detected a significant decrease of the ER−β density in the KO group compared to the WT group *: p<0,05 CB−1R+/+ vs CB−1R-/- group

Panel (E). ER−β density in the media layer. $n = 5$ (CB−1R+/+), $n = 10$ (CB−1R-/-). We detected a significant decrease of the ER−β density in the KO group compared to the WT group ***: p<0,001 CB−1R+/+ vs CB−1R-/- group

Panel (F). Representative photos of ER−β immunostained aorta segments, visualization with DAB on hematoxylin counterstaining, photographed by 10× magnification. Evaluation was performed from the values of the endothelial and the media layer. Statistical analysis was performed with unpaired *t*–test. Data were shown by noncalibrated optical density with mean ± SEM.

Panel C, F: Brown arrows shows the intima layer, and green arrows shows the media layer of the vessels.

4.2.4. Nitrative stress

There was no difference in the aortic walls between the WT and the CB−1R KO group NT density (Fig 12 A, B).

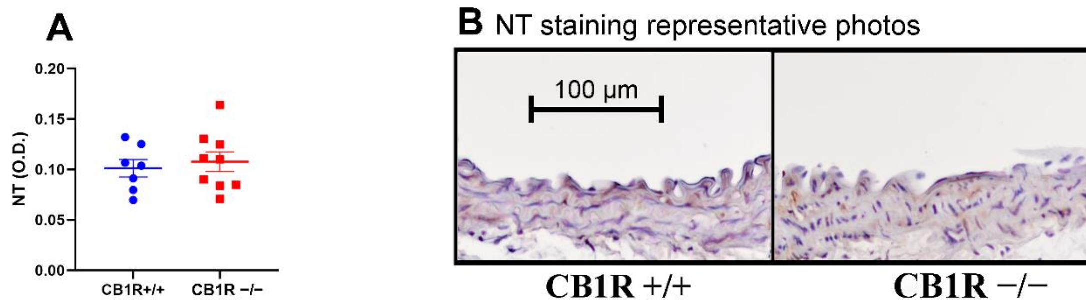

Figure 12. 3-nitrotirosine density of the abdominal aorta wall

Panel (A). Non−calibrated optical density data are shown with mean ± SEM, $n = 7$ (CB−1R+/+), $n = 9$ (CB−1R-/-). Statistical analysis performed with unpaired *t*–test.

Panel (B). Representative images of 3-nitrotyrosine staining. Visualization with DAB on hematoxylin counterstaining, photographed at 10× magnification. Evaluation was performed from the values of the media layer (115).

We summarized our results in Table 7.

Table 7: Summarizing table of the results from the CB-1 KO study (arrows show the modification effect of the knock out genotype.

Intima/media	↓
Intima diameter	-
Media diameter	↑
RF density	-
SMA density	-
endothelial eNOS	↑
endothelial COX-2	↓
TP	-
TXBAS	-
ERα	-
ERβ	↓
NT	-

5. DISCUSSION

In the first model, LPS treatment alone markedly compromised Ach-dependent vasorelaxation ability and substantially decreased cGMP levels in the vascular wall, which suggested reduced endothelium-dependent relaxation ability with reduced nitric oxide signal transduction in the smooth muscle cells, pointing to endothelial dysfunction (118, 119).

LPS also caused a significant decrease in the abundance of CB−1R and a tendency to decrease CB−2R. However, THC preserved the cardiovascular functions of LPS treated rats, decreased oxidative-nitrative stress, and the detectable amount of inducible cyclooxygenase. Moreover, the preserved endothelium-mediated relaxation was not dependent on the abundance of endothelial nitric oxide synthase or cGMP levels.

In the second model, we found that CB−1R deficiency induced morphological remodeling of the vascular (aortic) wall in female mice. Moreover, the CB−1R KO female mice showed a significant reduction in ER−β receptor density.

The density of eNOS was higher in CB−1R KO animals, indicating a role of endocannabinoids in regulating NOS expression in the aortic wall and regulating endothelial dependent dilation. COX−2 density in the aortic wall was lower in CB−1R KO mice. Thromboxane TP receptor density did not differ significantly between the two strains, but thromboxane synthase abundance tended to be higher in WT mice compared to KO mice.

5.1. Vascular effects of Delta-9-tetrahydrocannabinol or endocannabinoids and their signaling

THC therapy preserved Ach sensitivity in thoracic aortic rings impaired by LPS, suggesting a possible amelioration of LPS-induced endothelial dysfunction. Nonetheless, we observed a reduction in the abundance of both CB−1R and CB−2R in the aortic wall following the LPS challenge. We can find similar results from Navarro Dorado et al., who evaluated the impacts of chronic treatment of some mixed and selective CB agonists (WIN55 & JWH133) in a transgenic model of Alzheimer's disease. In their model, non-selective cannabinoid receptor activation reinstated the relaxation generated by acetylcholine (120). O'Sullivan et al. discovered that THC exhibited a time-dependent vasorelaxant effect, following an initial vasoconstrictor response, via PPAR-γ activation,

independent of CB−1R involvement in an in vitro rodent model (121). Stanley et al. demonstrated that cannabidiol (CBD), induces time-dependent vasorelaxation (vasorelaxation constantly increasing after CBD exposure) in human mesenteric arteries (from patient after colorectal surgery) (122). In the case of Stanley's study, this was facilitated directly by CB−1R and any other cannabinoid-dependent receptorial effects were excluded (CB−2R, PPAR-γ, Cyclooxygenase or other signaling pathways) (122). The cannabinoid system also regulates cerebral blood flow during hypoxia and hypercapnia – it can cause hypotension in a CB−1R dependent manner, and also can also cause hypertension in CB−1R independent manner (123). While previous studies evaluated the direct vasorelaxant properties of cannabinoid agonists, our research revealed the indirect vasoprotective benefits of THC. Similarly, other studies on streptozotocin-induced diabetic cardiomyopathy demonstrated the same outcomes with prolonged cannabis therapy. The vasorelaxation elicited by acetylcholine was also diminished in the streptozotocin-induced group, however THC successfully preserved this vasodilator function (52). The patterns of oxidative-nitrative stress aligned with our results: streptozotocin markedly elevated lipid peroxidation and nitrative stress indicators, which were normalized by THC (56, 124).

Vascular relaxation is regulated by the balance between vasoconstrictor and vasodilator mediators. The decreased detectability of COX−2 may indicate a decrease in the synthesis of thromboxane A2 (TXA2) in the THC treated cohort. Besides the shift in constrictor and relaxant prostanoids, we observed that nitric oxide (NO) levels remain stable – eNOS expression is reduced, but nitrotyrosine (NT) levels do not increase. This suggests that NO is not being further broken down, so any changes in vascular response may be linked to differences in the cGMP signaling pathway. Although both LPS-treated groups showed a decrease in cGMP density compared to the Control group, this decrease was only significant in the LPS-treated group, while the LPS+THC-treated group showed no significance. We can hypothesize that cannabinoids may also play a role in the change in NO mediated vasorelaxation. Although the literature on this topic is scarce – Bregg et al. found that cannabinoids have a guanylate cyclase influencing effect but this is not CB receptor dependent. (125). Further research is proposed to clarify the signal transduction of the THC induced better relaxation.

5.2. Anti-inflammatory effects of Delta-9-tetrahydrocannabinol

In our LPS induced model, we observed a decrease in aortic density of COX−2 and nitrative stress (detected by NT staining) in THC−treated rats.

Based on our results, the preserved endothelial function in THC treated endotoxic animals is partially due to better NO effect, shown by the slightly preserved cGMP immunopositivity in vascular smooth muscle cells. However, it is not the result of an increased expression of eNOS, as these animals had lower eNOS density. On the other hand, the lower nitrotyrosine content of these vessels suggest a better bioavailability of NO. During our experiments, we did not measure eNOS activity not have direct information about the rate of NO production. We also did not measure phosphodiesterase enzyme activity to determine the rate of cGMP degradation.

Another important pathway of endothelial function is the production of various prostanoid mediators. In inflammatory stated including LPS treatment the possible upregulation of COX-2 was reported. In our experiment we did not observe this increase. On the other hand, the LPS-THC group had lower COX-2 density compared to controls, suggesting a downregulation of COX-2 in these vessels. The consequently altered production of vasoactive prostanoids may also play role in the preserved endothelial function.

Both oxidative-nitrative stress and the upregulation of COX-2 is strongly connected to inflammatory processes, whereas the decline in these variables is usually connected to anti-inflammatory effects. The measured systemic oxidative stress marker MDA is also related to the activation of pro-inflammatory processes. LPS treatment significantly increased plasma MDA levels, which was prevented by THC co-treatment, suggesting an anti-inflammatory effect of THC. The anti-inflammatory effect of THC has been previously described. Joffre et al. (77) showed that THC treatment elevated the level of anti-inflammatory cytokine IL-10 while reduced the level of the pro-inflammatory CCL-2. They suggested the role of CB-1 receptor in this process. However, it is well known that immune cells express both CB-1 and CB-2 receptors being CB-2 the more abundant. CB2 signal transduction involves the inhibition of the NF-κB transcription factor, one of the central intracellular regulatory pathways of inflammation (126). The above-described anti-inflammatory effects of THC may play role in the reduced oxidative-nitrative stress and preserved vascular function in our experiments.

Moreover, preclinical and clinical research in conditions such as multiple sclerosis and inflammatory bowel disease indicate that THC-rich therapies reduce inflammatory biomarkers and enhance symptom scores while maintaining acceptable safety profiles. Suryavanshi et al. (127) and Szekely et al. both suggested the anti–inflammatory properties of THC the former on lipopolysaccharide-induced inflammatory response in human THP-1 macrophages and primary human bronchial epithelial cells (HBECs), the latter on LPS-challenged whole human blood cells (128).

5.3. Effects of exocannabinoids in endotoxemia or other pathological conditions

In our study we used the LPS dose of 5mg/kg (116) because our aim was to create a mild endotoxemic condition. To induce severe endotoxic shock, higher doses are implemented:
- inducing myocardial dysfunction Tian et al. used 40mg/kg LPS (129) or for lung injury Wang et al. used 12.5mg/kg body weight LPS (130). Our study used around eight-fold less compared to Tian et al. and around 40% of the dose used in the study of Wang et al. In comparison with previous studies, the endotoxemic state did not reach the level of endotoxin shock in our experiment.

CB−1R is highly expressed in cardiac and vascular cells, as well as in endothelial cells, and it is suggested to be a vascular smooth muscle receptor for cannabinoids (131). Our results indicate a decrease in the presence of cannabinoid receptors 1 and 2 in the aorta during endotoxemia. The downregulation of biologically available cannabinoid receptors may be due to decreased expression or increased receptor turnover. Receptor turnover can be regulated by β-arrestin2 binding, which leads to receptor internalization and cell desensitization (132, 133). Hunyady et al. discovered differences in the affinity of cannabinoid receptors 1 and 2 to β-arrestin2 in two different missense polymorphisms of CB−2R (132, 133). This suggests that the genetic presence of CB−2R (in wild-type or knock-out animals) may induce differences in cAMP levels and downstream MAPK/PI3K signaling, especially under prolonged stimulation, probably due to receptor internalization and trafficking (132, 133).

5.4. Role of Endocannabinoids and endocannabinoid receptors in the regulation of the vascular tone

It is well known that in healthy individuals exogenous phytocannabinoid exposition can cause severe health and cardiovascular diseases (134). Our second study showed that (115), in healthy female mice the lacking CB-1 receptor can improve the cardiovascular relaxation ability (e.g. enhanced eNOS density). Wei et al. also examined the effect of CB-1 receptor inhibition with peripherally selective CB−1 receptor antagonist on healthy vascular cells. In their experiment this blockage restored the inflammatory and endothelial cytotoxic effects of THC administration based on human umbilical vein cell culture experiments (135). Based on these results and in the light of the anti-inflammatory effect of CB−2 receptor activation (134), we conclude that CB-1 receptor overactivation may be the main cause of cardiovascular complications in juvenile marijuana users.

Previous studies have shown that cannabinoids and synthetic agonists, such as AEA, THC, and WIN 55,212-2, induce vasodilation in various vascular beds, including the aorta, coronary, and cerebral arteries (136-139). The vasodilatory effects of cannabinoids may vary depending on the type of blood vessel. Resistance arteries have been found to experience a greater relaxation effect than the aorta (136, 140). The initial vasodilatory and hypotensive effects may be facilitated by endocannabinoids upon stimulation of CB−1R. Most cannabinoid receptors are Gi/o protein-coupled receptors, therefore CB-1R activation on vascular smooth muscle cells may directly result in contraction. However, some studies observed vasodilator effects of exocannabinoids that cannot be explained with the above-mentioned mechanism. In vitro experiments showed that certain CB-1R agonists administered together with dopamine may act as inverse agonists of CB-1R reducing Gi/o signaling, therefore promoting the effect of Gs signal transduction and vasorelaxation. Another possibility is that vasorelaxant exocannabinoids act through non-canonical endocannabinoid receptors (141). Anandamide induced vasodilation was shown to involve NO production by vascular endothelial cells, which could be regulated by the calcium dependent activation of endothelial nitric oxide synthase. The responsible endocannabinoid receptor is still unclear; although CB-1R inverse agonist Rimonabant can inhibit the vasodilator effect, the connection between Gi/o and Ca signaling is still under investigation. AEA may also induce Ca signaling by binding to GPR55, which can couple either with Gq/11 or G12/13 G-proteins (142, 143).

Significant upregulation of eNOS expression were found in the CB−1R deficient group compared to the control animals. Similar effects found in wild−type conditions by Stanley

et al. that on healthy endothelial cells acute doseage of cannabidiol (CBD) significantly increased the phosphorylation of eNOS, thereby increasing their enzymatic activity (122). The use of the CB−1 receptor antagonist AM251 inhibited CBD-induced vasorelaxation. These results were confirmed by the use of the CB−1 receptor antagonist LY320135, which also reduced CBD-induced vasorelaxation significantly (122).

In a chronic pathological model on spontaneously hypertensive rats, inhibition of the anandamide degrading enzyme, fatty acid amide hydrolase (FAAH), by URB597, augmented AEA levels and improved vascular endothelial function in small mesenteric arteries (144). The present thesis investigates the acute and chronic, life-long vascular effects of CB−1R presence. Using this genetically modified line, we were able to identify several functions of the ECS in the control of vascular contraction-relaxation and vascular wall remodeling. We have found that vasodilator effects were augmented in CB−1R KO mice (115) (also published data in the co-author's thesis).

COX-2 plays an important role in degrading 2-AG. This can be a reason if somebody uses NSAID (nonsteroid anti-inflammatory drugs), it simultaneously increases 2-AG levels and thus indirectly affects the endocannabinoid system (145). On the other hand, if we see the phytocannabinoid side – several studies confirmed that phytocannabinoids e.g.: CBD via CB-2 signaling pathway can reduce COX-2 activity which consequently contributes to CB−2-dependent anti-inflammatory effects, and may also reduce the synthesis of the vasoactive prostanoid cocktail (146-148).

In both animal models, we observed a reduction in COX−2 levels, occurring both during stimulation of the endocannabinoid system with THC and during partial inhibition of the endocannabinoid system in our CB−1 receptor-deficient model. Considering that COX−2 participates in the synthesis of inflammatory and vasoactive prostanoids, it is significant that COX−2 also plays a role in the degradation of endocannabinoids (149).

We hypothesize that there may be different reasons for the decrease in density, although we cannot give a definitive answer because the literature is not clear on the subject.

The improved vasorelaxation observed in our LPS-induced model may be due to the anti-inflammatory effect of THC via CB−2 receptors, which is supported by the reduction in COX−2 (17).

However, other studies have suggested that THC and other cannabinoids exerts an indirect inhibitory impact on COX−2, (150) although this theory remains not sufficiently

clarified in the literature. Complex processes may underlie the reduction of COX−2 in our CB−1 receptor-deficient model. Some research suggests that there may be a crosstalk effect between the endocannabinoid system and COX−2 activity, where not only the CB−1 receptor is able to influence enzyme activity, but COX−2 is also able to produce endocannabinoid precursors in addition to the degradation of endocannabinoid AEA and 2-AG (150).

Additional study indicates that chronic inhibition of the CB−1 receptor may elevate levels of the vasodilator PGI2 while reducing levels of the vasoconstrictor TXB2 (17).

Based on these findings, we believe that the interactions between the cannabinoid system and COX−2 require further research and play an important role in both vasorelaxation and inflammatory processes. However, based on our results, we conclude that although there is no difference in TP receptor density, the reduced COX−2 density and the tendency of reduced thromboxane synthase in CB−1R KO mice may indicate that there is less TXA_2 or some other vasoconstrictor prostanoid. Therefore, it is suggested that the proportion of constrictor prostanoids in the "prostanoid cocktail" is relatively reduced in CB−1R-deficient state.

Our results show a significant reduction in intima-media ratio with media widening in the CB−1R deficient group compared to the control animals (Fig 7 and 8). The question remains whether this is an endocannabinoid dependent action or not. We observed a significant reduction in ER−β (estrogen receptor beta) density in the CB−1R deficient group, while ER−α density remained preserved. Therefore, it is hypothesized that a decrease in ER−β may affect the structural remodeling of the vascular wall. Even though vessel wall thickness increased in the receptor−deficient condition, our SMA staining showed no difference in the density of smooth muscle contractile proteins.

Despite the media layer thickening, we observed no difference in SMA density that was determined in association to the lack of CB−1 receptors. Several studies describe that inhibiting CB−1 receptors decreases SMA expression. These findings may explain why we could not observe any differences in smooth muscle density despite the thicker media layer (151, 152).

At the same time, we found no difference in elastic fiber density between the two genetic groups, indicating that elastin secretion by newly formed smooth muscle cells remained

unchanged. In his study, In pathological conditions Molica et al. described that CB−1R agonism promotes VSMC proliferation (153), while O'Leary et al. found no significant vascular wall remodeling with rimonabant (49). It is important to note that our study used healthy female mice, while Molica's study involved vessels that underwent balloon catheter injury and O'Leary examined vessel derived from a human study with obese patients (49).

The effects of cannabinoids and endocannabinoids on arterial function and remodeling may differ between normal and damaged vascular walls.

Inhibition of endocannabinoid degradation by FAAH inhibitor decreased the blood pressure of hypertensive rats (154). In humans, 30 mg of THC was observed to elevate blood pressure in normotensive healthy individuals, while an acute administration of a higher dosage of THC (600 mg) caused hypotension. In normotensive rats, WIN 55,212−2 increased blood pressure, while in hypertensive rats, the same CB−1 agonist reduced tension (155). Interestingly, based on Dol-Gleizes et al., the administration of CB−1R antagonist rimonabant (in an LDL receptor knock out strain) has been shown to reduce the risk of CVDs and to have anti-inflammatory effects, so it can reduce the development of atherosclerosis. On the other hand, as Pacher et al, and several other studies presented CB−2 receptor agonism also have anti-inflammatory effect (156-158).

5.5. Effect of the endocannabinoid system on nitrative stress levels

In both studies, the bioavailability of NO was assessed by eNOS and NT measurements. In endotoxemic rats THC reduced oxidative – nitrative stress. However, in our studies, we did not observe any changes in the nitration of vascular wall proteins in CB−1R KO animals.

The lack of CB-1 receptor increased the eNOS density, which is in accordance to the THC-induced results. Comparable findings are observed in the literature regarding the impact of other NOS enzymes. Jeon et al. also investigated inducible NOS gene expression in an LPS-induced model (159). Similar trends were observed in case of methamphetamine induced neurotoxicity in rats. In this case, concurrent THC treatment could protect against methamphetamine-induced neural NOS overexpression(160).

In the LPS+THC treated rats the reduced eNOS density and oxidative stress might both have contributed to the reduced NT staining. On the other hand, these changes in the NO

household did not alter the bioavailability of NO, as no changes in cGMP levels were observed in LPS+THC-treated animals.
However, the effect of the cannabinoid system on NO homeostasis is still not fully understood, as a review including cardioprotective effects suggests that there may even be an inducible NOS stimulatory effect in the heart (161).

5.6. Adaptation of the endocannabinoid system under different conditions

Based on our results, we conclude that under pathological (mild endotoxemic) conditions, THC treatment with appropriate purity and under in controlled conditions may have beneficial physiological effects that can be considered in addition to currently used approved therapeutic protocols.

However, in healthy groups, preventing the activation of the CB−1 receptor might be beneficial, since inhibition of the CB−1 receptor has been shown to have a favorable cardiovascular outcome.

Our study has shown that the cannabinoid system behaves differently in normal and pathological situations.

5.7. Limitations of the rodent models

Animal experiments, although represent a study on whole organism, with the interactions between cells, tissues and organs, have severe limitations: the (typically) inbred rodents have small genetic variety, and the housing ensures similar, close to optimal, low-stress environment (including the microbiome.) On the one hand, these circumstances allow lower case numbers. Despite their ubiquitousness rodent models usually poorly represents the human (or real-life veterinary) circumstances. The case numbers in both presented studies were below 10.
THC in endotoxemic rats
In the present research, only male rats were involved, although, according to the literature, man and women may have different responses to sepsis and THC. Relying on a single-dose LPS challenge creates an acute inflammatory state that lacks the complexity of true bacterial sepsis. There is no pathogen proliferation, biofilm formation or host–microbe interplay, which may exaggerate the benefits of THC. Simultaneous administration of LPS and THC further departs from clinical reality. Finally, measuring endpoints only

hours after treatment precludes insights into long-term cardiovascular outcomes and delayed adverse effects.

CB−1 Receptor Knockout in Female Mice

Using lifelong CB-1 receptor knockout mice provides clarity regarding receptor absence, but it also triggers compensatory developmental changes. Knockout animals lack both ligand binding and non-canonical receptor scaffolding or protein–protein interactions, whereas antagonists only block ligand access while preserving receptor structure. Focusing exclusively on females, highlights estrogen–endocannabinoid crosstalk, but limits the applicability to males or mixed-sex populations. While detailed analyses of aortic remodeling and isolated vessel reactivity provide mechanistic depth, they fail to capture systemic hemodynamics, behavioral effects or end-organ perfusion under stress. Furthermore, profiling only estrogen receptor ignores the broader hormonal milieu, including progesterone and adrenal steroids that can modulate vascular responses.

Clinical Translation Issues

When translating these findings into clinical practice, it is important to consider interspecies differences in cannabinoid metabolism and receptor distribution, given that rodent ECS dynamics often differ from those in humans. Genetic CB-1 ablation and pharmacological antagonism have different off-target and adaptive effects, so parallel studies using both approaches are needed. The experimental settings employed in these studies contrast sharply multifactorial progression of human disease. This highlights the importance of standardized dosing treatments, long-term safety evaluations and sex-inclusive clinical trials prior to the consideration of ECS-based therapies.

6. CONCLUSIONS

1. Δ^9-tetrahydrocannabinol improved endothelial vascular function and oxidative-nitrative stress in endotoxemic rats.

a.) THC treatment prevented endotoxemia induced endothel dysfunction.

THC treatment was effective in rescuing endothelial dysfunction caused by endotoxemia, so that Ach-dependent relaxation was significantly improved by THC treatment in LPS treated rats.

b.) The THC-treated rats' O/N stress markers were lower than in the LPS treated group and they did not differ from the control group (See on Table 6).

The anti-inflammatory effects of THC were also exerted - oxidative - nitrative stress levels were also significantly reduced (See on Table 6).

All these results lead us to conclude that THC or a similar phyto/exocannabinoid analogue offers favorable therapeutic options to reverse vascular damage in endotoxemia cases.

2. CB−1 receptor deficiency vessel morphological differences

a.) The abdominal aorta wall thickness significantly elevated while the intima media ratio decreased in the CB−1R$^{-/-}$ female mice, as we detected a significant media thickness elevation (See on Table 7).

b.) In the CB−1R$^{-/-}$ group, the density of enzymes contributing to relaxation in the vascular endothelium also changed: eNOS density increased, while COX−2 levels decreased.

c.) ER-α density did not differ but the ER-β density significantly decreased in the CB−1R$^{-/-}$ group (See on Table 7).

Based on these results we conclude that the favorable changes in nitric oxide and vasoactive prostanoid production together with morphological changes may contribute to an improved balance between vasoconstrictor and dilatory mechanisms.

7. SUMMARY

Both the endocannabinoid system (ECS) and estrogens play significant roles in cardiovascular control processes. Cannabinoid type 1 receptors mediate acute vasodilator and hypotensive effects, although their role in cardiovascular pathological conditions remains controversial. Estrogens are known to exert cardiovascular protection, particularly in females. This study aimed to explore the impact of ECS on vascular functions and the effects of the phytocannabinoid THC in endotoxemic model, and on the other hand, we also want to investigate the role of CB−1R deficiency in healthy female mice.

In a 24-hour endotoxemic rat model (E. coli-derived lipopolysaccharide, LPS, 5 mg/kg i.v.) animals were treated with THC (LPS+THC, 10 mg/kg i.p.). The endothelium-dependent relaxation of the thoracic aorta was measured using wire-myography. The molecular mechanisms of the vascular function were evaluated by measuring the density of eNOS, COX–2 and cGMP via immunohistochemistry. The oxidative – nitrative stress status has been defined as the density of oxidative stress marker 4-hydroxynonenal, the density of nitrative stress marker 3-nitrotyrosine, and the density poly(ADP-ribose) polymer. Further experiments were conducted on CB-1R knockout (CB1-R KO) and wild-type (WT) female mice. Abdominal aortas were isolated for histology. Histological analyses included hematoxylin-eosin and resorcin-fuchsin staining, as well as immunostainings for eNOS, COX-2, and estrogen receptors (ER−α, ER−β).

Results showed that endothelium-dependent relaxation was impaired by LPS but not in the LPS+THC group. THC treatment reduced oxidative-nitrative stress without affecting cGMP and eNOS density, suggesting an anti-inflammatory pathway activation. Histology revealed lower intima/media thickness and COX-2 density, higher eNOS, and lower ER-β density in CB1-R KO mice compared to WT mice.

These findings indicate that the absence of CB−1Rs in healthy females and the administration of THC in case of infection may have beneficial vascular effects, potentially mediated through anti-inflammatory pathways and enhanced endothelial NO utilization.

8. REFERENCES

1. Pertwee RG. Cannabinoid pharmacology: the first 66 years. Br J Pharmacol. 2006;147 Suppl 1(Suppl 1):S163-71.

2. Scherma M, Masia P, Satta V, Fratta W, Fadda P, Tanda G. Brain activity of anandamide: a rewarding bliss? Acta Pharmacol Sin. 2019;40(3):309-23.

3. Grabiec U, Dehghani F. N-Arachidonoyl Dopamine: A Novel Endocannabinoid and Endovanilloid with Widespread Physiological and Pharmacological Activities. Cannabis Cannabinoid Res. 2017;2(1):183-96.

4. Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol. 2005;16(5-6):297-313.

5. Maccarrone M, Finazzi-Agro A. The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death Differ. 2003;10(9):946-55.

6. Luchicchi A, Pistis M. Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. Mol Neurobiol. 2012;46(2):374-92.

7. Belali R, Mard SA, Khoshnam SE, Bavarsad K, Sarkaki A, Farbood Y. Anandamide improves food intake and orexinergic neuronal activity in the chronic sleep deprivation induction model in rats by modulating the expression of the CB1 receptor in the lateral hypothalamus. Neuropeptides. 2023;101:102336.

8. Kilaru A, Chapman KD. The endocannabinoid system. Essays Biochem. 2020;64(3):485-99.

9. Dziemitko S, Harasim-Symbor E, Chabowski A. How do phytocannabinoids affect cardiovascular health? An update on the most common cardiovascular diseases. Ther Adv Chronic Dis. 2023;14:20406223221143239.

10. Tallima H, El Ridi R. Arachidonic acid: Physiological roles and potential health benefits - A review. J Adv Res. 2018;11:33-41.

11. Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, et al. Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology. 2008;54(1):1-7.

12. Ueda N, Tsuboi K, Uyama T. N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). Prog Lipid Res. 2010;49(4):299-315.

13. Snider NT, Walker VJ, Hollenberg PF. Oxidation of the endogenous cannabinoid arachidonoyl ethanolamide by the cytochrome P450 monooxygenases: physiological and pharmacological implications. Pharmacol Rev. 2010;62(1):136-54.
14. Basavarajappa BS. Critical enzymes involved in endocannabinoid metabolism. Protein Pept Lett. 2007;14(3):237-46.
15. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14(12):1347-56.
16. Alhouayek M, Muccioli GG. COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. Trends Pharmacol Sci. 2014;35(6):284-92.
17. Burstein SH. Eicosanoid mediation of cannabinoid actions. Bioorg Med Chem. 2019;27(13):2718-28.
18. Rouzer CA, Marnett LJ. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. Chem Rev. 2011;111(10):5899-921.
19. Roque-Bravo R, Silva RS, Malheiro RF, Carmo H, Carvalho F, da Silva DD, et al. Synthetic Cannabinoids: A Pharmacological and Toxicological Overview. Annu Rev Pharmacol Toxicol. 2023;63:187-209.
20. Breijyeh Z, Jubeh B, Bufo SA, Karaman R, Scrano L. Cannabis: A Toxin-Producing Plant with Potential Therapeutic Uses. Toxins (Basel). 2021;13(2).
21. Howlett AC, Abood ME. CB(1) and CB(2) Receptor Pharmacology. Adv Pharmacol. 2017;80:169-206.
22. Pagano C, Navarra G, Coppola L, Avilia G, Bifulco M, Laezza C. Cannabinoids: Therapeutic Use in Clinical Practice. Int J Mol Sci. 2022;23(6).
23. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 2008;153(2):199-215.
24. Gregus AM, Buczynski MW. Druggable Targets in Endocannabinoid Signaling. Adv Exp Med Biol. 2020;1274:177-201.
25. Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc Natl Acad Sci U S A. 2008;105(7):2699-704.

26. de Almeida DL, Devi LA. Diversity of molecular targets and signaling pathways for CBD. Pharmacol Res Perspect. 2020;8(6):e00682.

27. Nigro E, Formato M, Crescente G, Daniele A. Cancer Initiation, Progression and Resistance: Are Phytocannabinoids from Cannabis sativa L. Promising Compounds? Molecules. 2021;26(9).

28. Schindler CW, Gramling BR, Justinova Z, Thorndike EB, Baumann MH. Synthetic cannabinoids found in "spice" products alter body temperature and cardiovascular parameters in conscious male rats. Drug Alcohol Depend. 2017;179:387-94.

29. Szabo R, Szabo Z, Borzsei D, Hoffmann A, Lesi ZN, Palszabo P, et al. Potential Implications of Rimonabant on Age-Related Oxidative Stress and Inflammation. Antioxidants (Basel). 2022;11(1).

30. Lowe H, Toyang N, Steele B, Bryant J, Ngwa W. The Endocannabinoid System: A Potential Target for the Treatment of Various Diseases. Int J Mol Sci. 2021;22(17).

31. Pintori N, Caria F, De Luca MA, Miliano C. THC and CBD: Villain versus Hero? Insights into Adolescent Exposure. Int J Mol Sci. 2023;24(6).

32. Banana Y, Bashir H, Boukabous S, Rezziki A, Benzirar A, El Mahi O. Cannabis arteritis: A case report and brief review of the literature. Ann Med Surg (Lond). 2022;76:103523.

33. Pacher P, Steffens S, Hasko G, Schindler TH, Kunos G. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. Nat Rev Cardiol. 2018;15(3):151-66.

34. Subramaniam VN, Menezes AR, DeSchutter A, Lavie CJ. The Cardiovascular Effects of Marijuana: Are the Potential Adverse Effects Worth the High? Mo Med. 2019;116(2):146-53.

35. Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. N Engl J Med. 2014;370(23):2219-27.

36. Panlilio LV, Goldberg SR, Justinova Z. Cannabinoid abuse and addiction: Clinical and preclinical findings. Clin Pharmacol Ther. 2015;97(6):616-27.

37. Zehra A, Burns J, Liu CK, Manza P, Wiers CE, Volkow ND, et al. Cannabis Addiction and the Brain: a Review. J Neuroimmune Pharmacol. 2018;13(4):438-52.

38. Prud'homme M, Cata R, Jutras-Aswad D. Cannabidiol as an Intervention for Addictive Behaviors: A Systematic Review of the Evidence. Subst Abuse. 2015;9:33-8.

39. Cooper ZD. Adverse Effects of Synthetic Cannabinoids: Management of Acute Toxicity and Withdrawal. Curr Psychiatry Rep. 2016;18(5):52.

40. van Amsterdam J, Brunt T, van den Brink W. The adverse health effects of synthetic cannabinoids with emphasis on psychosis-like effects. J Psychopharmacol. 2015;29(3):254-63.

41. Crean RD, Crane NA, Mason BJ. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. J Addict Med. 2011;5(1):1-8.

42. Malinowska B, Baranowska-Kuczko M, Schlicker E. Triphasic blood pressure responses to cannabinoids: do we understand the mechanism? Br J Pharmacol. 2012;165(7):2073-88.

43. Sharma V, Bhardwaj A, Sahai A, Singh S, Shamim S. Myocardial Infarction Triggered by Marijuana Use. JACC Case Rep. 2025;30(8):103202.

44. Liu D, Yang L, Liu P, Wang Y, Gao L. Impact of cannabis abuse on the occurrence of stroke in young people: a systematic review and meta-analysis. Front Neurol. 2024;15:1426023.

45. El Omri N, Eljaoudi R, Mekouar F, Jira M, Sekkach Y, Amezyane T, et al. Cannabis arteritis. Pan Afr Med J. 2017;26:53.

46. Kaniecki T, Huso TH, Haque UJ. Cannabis arteritis revisited: a case report with distinct pathologic features and review of the literature. Rheumatology (Oxford). 2023.

47. Pilitsi E, Kennamer B, Trepanowski N, Gonzalez R, Trojanowski M, Phillips T, et al. Cannabis arteritis presenting with Raynaud's and digital ulcerations: a case-based review of a controversial thromboangiitis obliterans-like condition. Clin Rheumatol. 2023;42(7):1981-5.

48. Goyal H, Awad HH, Ghali JK. Role of cannabis in cardiovascular disorders. J Thorac Dis. 2017;9(7):2079-92.

49. O'Leary DH, Reuwer AQ, Nissen SE, Despres JP, Deanfield JE, Brown MW, et al. Effect of rimonabant on carotid intima-media thickness (CIMT) progression in patients with abdominal obesity and metabolic syndrome: the AUDITOR Trial. Heart. 2011;97(14):1143-50.

50. Cheung CP, Coates AM, Baker RE, Burr JF. Acute Effects of Cannabis Inhalation on Arterial Stiffness, Vascular Endothelial Function, and Cardiac Function. J Am Heart Assoc. 2024;13(23):e037731.
51. Pergolizzi JV, Jr., Lequang JA, Taylor R, Jr., Raffa RB, Colucci D, Group NR. The role of cannabinoids in pain control: the good, the bad, and the ugly. Minerva Anestesiol. 2018;84(8):955-69.
52. Kiskova T, Mungenast F, Suvakova M, Jager W, Thalhammer T. Future Aspects for Cannabinoids in Breast Cancer Therapy. Int J Mol Sci. 2019;20(7).
53. Xiong X, Chen S, Shen J, You H, Yang H, Yan C, et al. Cannabis suppresses antitumor immunity by inhibiting JAK/STAT signaling in T cells through CNR2. Signal Transduct Target Ther. 2022;7(1):99.
54. Baranowska-Kuczko M, Kozlowska H, Kloza M, Kusaczuk M, Harasim-Symbor E, Biernacki M, et al. Vasoprotective Endothelial Effects of Chronic Cannabidiol Treatment and Its Influence on the Endocannabinoid System in Rats with Primary and Secondary Hypertension. Pharmaceuticals (Basel). 2021;14(11).
55. Meah F, Lundholm M, Emanuele N, Amjed H, Poku C, Agrawal L, et al. The effects of cannabis and cannabinoids on the endocrine system. Rev Endocr Metab Disord. 2022;23(3):401-20.
56. Vella RK, Jackson DJ, Fenning AS. Delta(9)-Tetrahydrocannabinol Prevents Cardiovascular Dysfunction in STZ-Diabetic Wistar-Kyoto Rats. Biomed Res Int. 2017;2017:7974149.
57. Stanley C, O'Sullivan SE. Vascular targets for cannabinoids: animal and human studies. Br J Pharmacol. 2014;171(6):1361-78.
58. Watanabe AH, Navaravong L, Sirilak T, Prasitwarachot R, Nathisuwan S, Page RL, 2nd, et al. A systematic review and meta-analysis of randomized controlled trials of cardiovascular toxicity of medical cannabinoids. J Am Pharm Assoc (2003). 2021;61(5):e1-e13.
59. Hu J, Fan W, Xu Y, Li X, Zhang H, Li S, et al. Maladaptive changes in the homeostasis of AEA-TRPV1/CB1R induces pain-related hyperactivity of nociceptors after spinal cord injury. Cell Biosci. 2025;15(1):2.
60. Iannotti FA, Vitale RM. The Endocannabinoid System and PPARs: Focus on Their Signalling Crosstalk, Action and Transcriptional Regulation. Cells. 2021;10(3).

61. Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, et al. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. Proc Natl Acad Sci U S A. 1999;96(24):14136-41.
62. Vallejos A, Olivares P, Gatica S, Villegas V, Echeverria C, Cabello-Verrugio C, et al. Endotoxemia-induced endothelial fibrosis inhibition improves hypotension, tachycardia, multiple organ dysfunction syndrome, cytokine response, oxidative stress, and survival. Lab Invest. 2019;99(8):1173-92.
63. Hinder F, Stubbe HD, Van Aken H, Baba HA, Jahn UR, Brodner G, et al. Early multiple organ failure after recurrent endotoxemia in the presence of vasoconstrictor-masked hypovolemia. Crit Care Med. 2003;31(3):903-9.
64. Roul D, Rozec B, Andre G, Merlet N, Tran Quang T, Lauzier B, et al. Increased beta2-adrenergic vasorelaxation at the early phase of endotoxemic shock in rats. Vascul Pharmacol. 2015;72:181-9.
65. Umans JG, Wylam ME, Samsel RW, Edwards J, Schumacker PT. Effects of endotoxin in vivo on endothelial and smooth-muscle function in rabbit and rat aorta. Am Rev Respir Dis. 1993;148(6 Pt 1):1638-45.
66. Ischiropoulos H, al-Mehdi AB. Peroxynitrite-mediated oxidative protein modifications. FEBS Lett. 1995;364(3):279-82.
67. Masszi G, Horvath EM, Tarszabo R, Benko R, Novak A, Buday A, et al. Reduced estradiol-induced vasodilation and poly-(ADP-ribose) polymerase (PARP) activity in the aortas of rats with experimental polycystic ovary syndrome (PCOS). PloS one. 2013;8(3):e55589.
68. Sido JM, Nagarkatti PS, Nagarkatti M. Delta(9)-Tetrahydrocannabinol attenuates allogeneic host-versus-graft response and delays skin graft rejection through activation of cannabinoid receptor 1 and induction of myeloid-derived suppressor cells. J Leukoc Biol. 2015;98(3):435-47.
69. Henshaw FR, Dewsbury LS, Lim CK, Steiner GZ. The Effects of Cannabinoids on Pro- and Anti-Inflammatory Cytokines: A Systematic Review of In Vivo Studies. Cannabis Cannabinoid Res. 2021;6(3):177-95.
70. Burstein S. Molecular Mechanisms for the Inflammation-Resolving Actions of Lenabasum. Mol Pharmacol. 2021;99(2):125-32.

71. Onaivi ES, Sharma V. Cannabis for COVID-19: can cannabinoids quell the cytokine storm? Future Sci OA. 2020;6(8):FSO625.
72. Rossi F, Tortora C, Argenziano M, Di Paola A, Punzo F. Cannabinoid Receptor Type 2: A Possible Target in SARS-CoV-2 (CoV-19) Infection? Int J Mol Sci. 2020;21(11).
73. Toguri JT, Moxsom R, Szczesniak AM, Zhou J, Kelly ME, Lehmann C. Cannabinoid 2 receptor activation reduces leukocyte adhesion and improves capillary perfusion in the iridial microvasculature during systemic inflammation. Clin Hemorheol Microcirc. 2015;61(2):237-49.
74. Kapellos TS, Recio C, Greaves DR, Iqbal AJ. Cannabinoid Receptor 2 Modulates Neutrophil Recruitment in a Murine Model of Endotoxemia. Mediators Inflamm. 2017;2017:4315412.
75. Toguri JT, Lehmann C, Laprairie RB, Szczesniak AM, Zhou J, Denovan-Wright EM, et al. Anti-inflammatory effects of cannabinoid CB(2) receptor activation in endotoxin-induced uveitis. Br J Pharmacol. 2014;171(6):1448-61.
76. Turcotte C, Blanchet MR, Laviolette M, Flamand N. The CB(2) receptor and its role as a regulator of inflammation. Cell Mol Life Sci. 2016;73(23):4449-70.
77. Joffre J, Yeh CC, Wong E, Thete M, Xu F, Zlatanova I, et al. Activation of CB(1)R Promotes Lipopolysaccharide-Induced IL-10 Secretion by Monocytic Myeloid-Derived Suppressive Cells and Reduces Acute Inflammation and Organ Injury. J Immunol. 2020;204(12):3339-50.
78. El-Talatini MR, Taylor AH, Konje JC. Fluctuation in anandamide levels from ovulation to early pregnancy in in-vitro fertilization-embryo transfer women, and its hormonal regulation. Hum Reprod. 2009;24(8):1989-98.
79. Karasu T, Marczylo TH, Maccarrone M, Konje JC. The role of sex steroid hormones, cytokines and the endocannabinoid system in female fertility. Hum Reprod Update. 2011;17(3):347-61.
80. Bari M, Battista N, Pirazzi V, Maccarrone M. The manifold actions of endocannabinoids on female and male reproductive events. Front Biosci (Landmark Ed). 2011;16(2):498-516.
81. Brents LK. Marijuana, the Endocannabinoid System and the Female Reproductive System. Yale J Biol Med. 2016;89(2):175-91.

82. Walker OS, Holloway AC, Raha S. The role of the endocannabinoid system in female reproductive tissues. J Ovarian Res. 2019;12(1):3.

83. du Plessis SS, Agarwal A, Syriac A. Marijuana, phytocannabinoids, the endocannabinoid system, and male fertility. J Assist Reprod Genet. 2015;32(11):1575-88.

84. Cui N, Feng X, Zhao Z, Zhang J, Xu Y, Wang L, et al. Restored Plasma Anandamide and Endometrial Expression of Fatty Acid Amide Hydrolase in Women With Polycystic Ovary Syndrome by the Combination Use of Diane-35 and Metformin. Clin Ther. 2017;39(4):751-8.

85. Cui N, Yang Y, Xu Y, Zhang J, Jiang L, Hao G. Decreased expression of fatty acid amide hydrolase in women with polycystic ovary syndrome. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2017;33(5):368-72.

86. Juan CC, Chen KH, Wang PH, Hwang JL, Seow KM. Endocannabinoid system activation may be associated with insulin resistance in women with polycystic ovary syndrome. Fertil Steril. 2015;104(1):200-6.

87. Maccarrone M, Bari M, Di Rienzo M, Finazzi-Agro A, Rossi A. Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin. J Biol Chem. 2003;278(35):32726-32.

88. Balint F, Liposits Z, Farkas I. Estrogen Receptor Beta and 2-arachidonoylglycerol Mediate the Suppressive Effects of Estradiol on Frequency of Postsynaptic Currents in Gonadotropin-Releasing Hormone Neurons of Metestrous Mice: An Acute Slice Electrophysiological Study. Front Cell Neurosci. 2016;10:77.

89. Cecconi S, Rossi G, Castellucci A, D'Andrea G, Maccarrone M. Endocannabinoid signaling in mammalian ovary. Eur J Obstet Gynecol Reprod Biol. 2014;178:6-11.

90. Cecconi S, Rossi G, Oddi S, Di Nisio V, Maccarrone M. Role of Major Endocannabinoid-Binding Receptors during Mouse Oocyte Maturation. Int J Mol Sci. 2019;20(12).

91. Alvarez S. Do some addictions interfere with fertility? Fertil Steril. 2015;103(1):22-6.

92. Park B, McPartland JM, Glass M. Cannabis, cannabinoids and reproduction. Prostaglandins Leukot Essent Fatty Acids. 2004;70(2):189-97.

93. Wang H, Dey SK, Maccarrone M. Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. Endocr Rev. 2006;27(5):427-48.

94. Taylor AH, Abbas MS, Habiba MA, Konje JC. Histomorphometric evaluation of cannabinoid receptor and anandamide modulating enzyme expression in the human endometrium through the menstrual cycle. Histochem Cell Biol. 2010;133(5):557-65.

95. Amantea D, Spagnuolo P, Bari M, Fezza F, Mazzei C, Tassorelli C, et al. Modulation of the endocannabinoid system by focal brain ischemia in the rat is involved in neuroprotection afforded by 17beta-estradiol. FEBS J. 2007;274(17):4464-775.

96. El-Talatini MR, Taylor AH, Elson JC, Brown L, Davidson AC, Konje JC. Localisation and function of the endocannabinoid system in the human ovary. PloS one. 2009;4(2):e4579.

97. Darblade B, Pendaries C, Krust A, Dupont S, Fouque MJ, Rami J, et al. Estradiol alters nitric oxide production in the mouse aorta through the alpha-, but not beta-, estrogen receptor. Circ Res. 2002;90(4):413-9.

98. Savva C, Korach-Andre M. Estrogen Receptor beta (ERbeta) Regulation of Lipid Homeostasis-Does Sex Matter? Metabolites. 2020;10(3).

99. Aryan L, Younessi D, Zargari M, Banerjee S, Agopian J, Rahman S, et al. The Role of Estrogen Receptors in Cardiovascular Disease. Int J Mol Sci. 2020;21(12).

100. Iorga A, Umar S, Ruffenach G, Aryan L, Li J, Sharma S, et al. Estrogen rescues heart failure through estrogen receptor Beta activation. Biol Sex Differ. 2018;9(1):48.

101. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERalpha) and beta (ERbeta): subtype-selective ligands and clinical potential. Steroids. 2014;90:13-29.

102. Bailone RL, Fukushima HCS, de Aguiar LK, Borra RC. The endocannabinoid system in zebrafish and its potential to study the effects of Cannabis in humans. Lab Anim Res. 2022;38(1):5.

103. Lachowicz J, Szopa A, Ignatiuk K, Swiader K, Serefko A. Zebrafish as an Animal Model in Cannabinoid Research. Int J Mol Sci. 2023;24(13).

104. Panlilio LV, Justinova Z, Goldberg SR. Animal models of cannabinoid reward. Br J Pharmacol. 2010;160(3):499-510.

105. Greco R, Gasperi V, Sandrini G, Bagetta G, Nappi G, Maccarrone M, et al. Alterations of the endocannabinoid system in an animal model of migraine: evaluation in cerebral areas of rat. Cephalalgia. 2010;30(3):296-302.
106. Kogan NM, Begmatova D, Vinnikova L, Malitsky S, Itkin M, Sharon E, et al. Endocannabinoid basis of personality-Insights from animal model of social behavior. Front Pharmacol. 2023;14:1234332.
107. Woods SC. Role of the endocannabinoid system in regulating cardiovascular and metabolic risk factors. Am J Med. 2007;120(3 Suppl 1):S19-25.
108. Cavuoto P, McAinch AJ, Hatzinikolas G, Janovska A, Game P, Wittert GA. The expression of receptors for endocannabinoids in human and rodent skeletal muscle. Biochem Biophys Res Commun. 2007;364(1):105-10.
109. Strittmatter F, Gandaglia G, Benigni F, Bettiga A, Rigatti P, Montorsi F, et al. Expression of fatty acid amide hydrolase (FAAH) in human, mouse, and rat urinary bladder and effects of FAAH inhibition on bladder function in awake rats. Eur Urol. 2012;61(1):98-106.
110. Mukherjee S, Adams M, Whiteaker K, Daza A, Kage K, Cassar S, et al. Species comparison and pharmacological characterization of rat and human CB2 cannabinoid receptors. Eur J Pharmacol. 2004;505(1-3):1-9.
111. Gutierrez A, Creehan KM, Turner ML, Tran RN, Kerr TM, Nguyen JD, et al. Vapor exposure to Delta9-tetrahydrocannabinol (THC) slows locomotion of the Maine lobster (Homarus americanus). Pharmacol Biochem Behav. 2021;207:173222.
112. Park SH, Koch S, Richardson K, Pauli C, Han JH, Kwon TH. Tobacco Hornworm as an Insect Model System for Cannabinoid Pre-clinical Studies. J Vis Exp. 2021(178).
113. Silver RJ. The Endocannabinoid System of Animals. Animals (Basel). 2019;9(9).
114. Rosado-Franco JJ, Ellison AL, White CJ, Price AS, Moore CF, Williams RE, et al. Roadmap for the expression of canonical and extended endocannabinoid system receptors and metabolic enzymes in peripheral organs of preclinical animal models. Physiol Rep. 2024;12(4):e15947.
115. Banyai B, Vass Z, Kiss S, Balogh A, Brandhuber D, Karvaly G, et al. Role of CB1 Cannabinoid Receptors in Vascular Responses and Vascular Remodeling of the Aorta in Female Mice. Int J Mol Sci. 2023;24(22).

116. Banyai B, Repas C, Miklos Z, Johnsen J, Horvath EM, Benko R. Delta 9-tetrahydrocannabinol conserves cardiovascular functions in a rat model of endotoxemia: Involvement of endothelial molecular mechanisms and oxidative-nitrative stress. PloS one. 2023;18(6):e0287168.

117. Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A. 1999;96(10):5780-5.

118. Wang M, Feng J, Zhou D, Wang J. Bacterial lipopolysaccharide-induced endothelial activation and dysfunction: a new predictive and therapeutic paradigm for sepsis. Eur J Med Res. 2023;28(1):339.

119. Bannerman DD, Goldblum SE. Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. Am J Physiol Lung Cell Mol Physiol. 2003;284(6):L899-914.

120. Navarro-Dorado J, Villalba N, Prieto D, Brera B, Martin-Moreno AM, Tejerina T, et al. Vascular Dysfunction in a Transgenic Model of Alzheimer's Disease: Effects of CB1R and CB2R Cannabinoid Agonists. Front Neurosci. 2016;10:422.

121. O'Sullivan SE, Tarling EJ, Bennett AJ, Kendall DA, Randall MD. Novel time-dependent vascular actions of Delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. Biochem Biophys Res Commun. 2005;337(3):824-31.

122. Stanley CP, Hind WH, Tufarelli C, O'Sullivan SE. Cannabidiol causes endothelium-dependent vasorelaxation of human mesenteric arteries via CB1 activation. Cardiovasc Res. 2015;107(4):568-78.

123. Iring A, Ruisanchez E, Leszl-Ishiguro M, Horvath B, Benko R, Lacza Z, et al. Role of endocannabinoids and cannabinoid-1 receptors in cerebrocortical blood flow regulation. PloS one. 2013;8(1):e53390.

124. Rajesh M, Mukhopadhyay P, Batkai S, Patel V, Saito K, Matsumoto S, et al. Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. J Am Coll Cardiol. 2010;56(25):2115-25.

125. Begg M, Mo FM, Offertaler L, Batkai S, Pacher P, Razdan RK, et al. G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca2+-dependent K+ current. J Biol Chem. 2003;278(46):46188-94.
126. Anil SM, Peeri H, Koltai H. Medical Cannabis Activity Against Inflammation: Active Compounds and Modes of Action. Front Pharmacol. 2022;13:908198.
127. Suryavanshi SV, Zaiachuk M, Pryimak N, Kovalchuk I, Kovalchuk O. Cannabinoids Alleviate the LPS-Induced Cytokine Storm via Attenuating NLRP3 Inflammasome Signaling and TYK2-Mediated STAT3 Signaling Pathways In Vitro. Cells. 2022;11(9).
128. Szekely Y, Ingbir M, Bentur OS, Hochner O, Porat R. Natural cannabinoids suppress the cytokine storm in sepsis-like in vitro model. Eur Cytokine Netw. 2020;31(2):50-8.
129. Tian W, Liu SY, Zhang M, Meng JR, Tang N, Feng YD, et al. TRPC1 contributes to endotoxemia-induced myocardial dysfunction via mediating myocardial apoptosis and autophagy. Pharmacol Res. 2022;181:106262.
130. Wang L, Cao Y, Gorshkov B, Zhou Y, Yang Q, Xu J, et al. Ablation of endothelial Pfkfb3 protects mice from acute lung injury in LPS-induced endotoxemia. Pharmacol Res. 2019;146:104292.
131. Lepicier P, Lagneux C, Sirois MG, Lamontagne D. Endothelial CB1-receptors limit infarct size through NO formation in rat isolated hearts. Life Sci. 2007;81(17-18):1373-80.
132. Gyombolai P, Boros E, Hunyady L, Turu G. Differential beta-arrestin2 requirements for constitutive and agonist-induced internalization of the CB1 cannabinoid receptor. Mol Cell Endocrinol. 2013;372(1-2):116-27.
133. Turu G, Soltesz-Katona E, Toth AD, Juhasz C, Cserzo M, Misak A, et al. Biased Coupling to beta-Arrestin of Two Common Variants of the CB(2) Cannabinoid Receptor. Frontiers in endocrinology. 2021;12:714561.
134. Wang A, Dey S, Subhan S, Patel J, Frishman WH, Aronow WS. Cardiovascular Effects of Cannabinoids. Cardiol Rev. 2023.
135. Wei TT, Chandy M, Nishiga M, Zhang A, Kumar KK, Thomas D, et al. Cannabinoid receptor 1 antagonist genistein attenuates marijuana-induced vascular inflammation. Cell. 2022;185(10):1676-93 e23.

136. Dannert MT, Alsasua A, Herradon E, Martin MI, Lopez-Miranda V. Vasorelaxant effect of Win 55,212-2 in rat aorta: new mechanisms involved. Vascul Pharmacol. 2007;46(1):16-23.

137. O'Sullivan SE, Randall MD, Gardiner SM. The in vitro and in vivo cardiovascular effects of Delta9-tetrahydrocannabinol in rats made hypertensive by chronic inhibition of nitric-oxide synthase. J Pharmacol Exp Ther. 2007;321(2):663-72.

138. Randall MD, Kendall DA, O'Sullivan S. The complexities of the cardiovascular actions of cannabinoids. Br J Pharmacol. 2004;142(1):20-6.

139. Wagner JA, Jarai Z, Batkai S, Kunos G. Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB(1) receptors. Eur J Pharmacol. 2001;423(2-3):203-10.

140. White R, Hiley CR. The actions of some cannabinoid receptor ligands in the rat isolated mesenteric artery. Br J Pharmacol. 1998;125(3):533-41.

141. Eldeeb K, Leone-Kabler S, Howlett AC. CB1 cannabinoid receptor-mediated increases in cyclic AMP accumulation are correlated with reduced Gi/o function. J Basic Clin Physiol Pharmacol. 2016;27(3):311-22.

142. Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. J Mol Endocrinol. 2010;44(2):75-85.

143. Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, et al. Integrin clustering enables anandamide-induced Ca2+ signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. J Cell Sci. 2008;121(Pt 10):1704-17.

144. Baranowska-Kuczko M, Kozlowska H, Kloza M, Harasim-Symbor E, Biernacki M, Kasacka I, et al. Beneficial Changes in Rat Vascular Endocannabinoid System in Primary Hypertension and under Treatment with Chronic Inhibition of Fatty Acid Amide Hydrolase by URB597. Int J Mol Sci. 2021;22(9).

145. Paunescu H, Coman OA, Coman L, Ghita I, Georgescu SR, Draghia F, et al. Cannabinoid system and cyclooxygenases inhibitors. J Med Life. 2011;4(1):11-20.

146. Morgan AJ, Kingsley PJ, Mitchener MM, Altemus M, Patrick TA, Gaulden AD, et al. Detection of Cyclooxygenase-2-Derived Oxygenation Products of the Endogenous Cannabinoid 2-Arachidonoylglycerol in Mouse Brain. ACS Chem Neurosci. 2018;9(7):1552-9.

147. Pellati F, Borgonetti V, Brighenti V, Biagi M, Benvenuti S, Corsi L. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. Biomed Res Int. 2018;2018:1691428.
148. Cosentino M, Legnaro M, Luini A, Ferrari M, Sodergren M, Pacchetti B, et al. Effect of Cannabidiol on Cyclooxygenase Type 1 and 2 Expression and Function in Human Neutrophils. Cannabis Cannabinoid Res. 2023;8(6):999-1007.
149. Kozak KR, Prusakiewicz JJ, Marnett LJ. Oxidative metabolism of endocannabinoids by COX-2. Curr Pharm Des. 2004;10(6):659-67.
150. Stachowicz K. Deciphering the mechanisms of reciprocal regulation or interdependence at the cannabinoid CB1 receptors and cyclooxygenase-2 level: Effects on mood, cognitive implications, and synaptic signaling. Neurosci Biobehav Rev. 2023;155:105439.
151. Greiner B, Sommerfeld M, Kintscher U, Unger T, Kappert K, Kaschina E. Differential Regulation of MMPs, Apoptosis and Cell Proliferation by the Cannabinoid Receptors CB1 and CB2 in Vascular Smooth Muscle Cells and Cardiac Myocytes. Biomedicines. 2022;10(12).
152. Tiyerili V, Zimmer S, Jung S, Wassmann K, Naehle CP, Lutjohann D, et al. CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. Basic Res Cardiol. 2010;105(4):465-77.
153. Molica F, Burger F, Thomas A, Staub C, Tailleux A, Staels B, et al. Endogenous cannabinoid receptor CB1 activation promotes vascular smooth-muscle cell proliferation and neointima formation. J Lipid Res. 2013;54(5):1360-8.
154. Batkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, et al. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation. 2004;110(14):1996-2002.
155. Ho WS, Gardiner SM. Acute hypertension reveals depressor and vasodilator effects of cannabinoids in conscious rats. Br J Pharmacol. 2009;156(1):94-104.
156. Dobovisek L, Hojnik M, Ferk P. Overlapping molecular pathways between cannabinoid receptors type 1 and 2 and estrogens/androgens on the periphery and their involvement in the pathogenesis of common diseases (Review). Int J Mol Med. 2016;38(6):1642-51.

157. Dol-Gleizes F, Paumelle R, Visentin V, Mares AM, Desitter P, Hennuyer N, et al. Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2009;29(1):12-8.
158. Pacher P. Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? Arterioscler Thromb Vasc Biol. 2009;29(1):7-9.
159. Jeon YJ, Yang KH, Pulaski JT, Kaminski NE. Attenuation of inducible nitric oxide synthase gene expression by delta 9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor- kappa B/Rel activation. Mol Pharmacol. 1996;50(2):334-41.
160. Castelli MP, Madeddu C, Casti A, Casu A, Casti P, Scherma M, et al. Delta9-tetrahydrocannabinol prevents methamphetamine-induced neurotoxicity. PloS one. 2014;9(5):e98079.
161. Maslov LN, Khaliulin I, Zhang Y, Krylatov AV, Naryzhnaya NV, Mechoulam R, et al. Prospects for Creation of Cardioprotective Drugs Based on Cannabinoid Receptor Agonists. J Cardiovasc Pharmacol Ther. 2016;21(3):262-72.

9. BIBLIOGRAPHY OF PUBLICATIONS

Publications related to the thesis:

1. Banyai B, Repas C, Miklos Z, Johnsen J, Horvath EM, Benko R. Delta 9-tetrahydrocannabinol conserves cardiovascular functions in a rat model of endotoxemia: Involvement of endothelial molecular mechanisms and oxidative-nitrative stress. PLoS One. 2023;18(6):e0287168.

IF: 2.9

2. Banyai B, Vass Z, Kiss S, Balogh A, Brandhuber D, Karvaly G, et al. Role of CB1 Cannabinoid Receptors in Vascular Responses and Vascular Remodeling of the Aorta in Female Mice. Int J Mol Sci. 2023;24(22).

IF: 4.9

Publications not related to the thesis:

3. Sziva RE, Kollarics R, Pal E, Banyai B, Korsos-Novak A, Fontanyi Z, et al. Increased Oxidative and Nitrative Stress and Decreased Sex Steroid Relaxation in a Vitamin D-Deficient Hyperandrogenic Rodent Model-And a Validation of the Polycystic Ovary Syndrome Model. Nutrients. 2025;17(2).

IF: 5.0

4. Vass Z, Shenker-Horvath K, Banyai B, Veto KN, Torok V, Gem JB, et al. Investigating the Role of Cannabinoid Type 1 Receptors in Vascular Function and Remodeling in a Hypercholesterolemic Mouse Model with Low-Density Lipoprotein-Cannabinoid Type 1 Receptor Double Knockout Animals. Int J Mol Sci. 2024;25(17).

IF: 4.9

5. Suli A, Magyar P, Vezer M, Banyai B, Szekeres M, Sipos M, et al. Effects of Gender and Vitamin D on Vascular Reactivity of the Carotid Artery on a Testosterone-Induced PCOS Model. Int J Mol Sci. 2023;24(23).

IF: 4.9

6. Bencsics M, Banyai B, Ke H, Csepanyi-Komi R, Sasvari P, Dantzer F, et al. PARP2 downregulation in T cells ameliorates lipopolysaccharide-induced inflammation of the large intestine. Front Immunol. 2023;14:1135410.

IF: 5.7

7. Vezer M, Josvai A, Banyai B, Acs N, Keszthelyi M, Soltesz-Katona E, et al. Impact of Sex and Exercise on Femoral Artery Function: More Favorable Adaptation in Male Rats. Life (Basel). 2023;13(3).

IF: 3.2

8. Vezer M, Demeter A, Szekeres M, Josvai A, Banyai B, Olah A, et al. Sex differences in rat renal arterial responses following exercise training. Am J Physiol Heart Circ Physiol. 2022;322(2):H310-H8.

IF: 4.8

9. Tarszabo R, Banyai B, Ruisanchez E, Peterffy B, Korsos-Novak A, Lajtai K, et al. Influence of Vitamin D on the Vasoactive Effect of Estradiol in a Rat Model of Polycystic Ovary Syndrome. Int J Mol Sci. 2021;22(17).

IF: 6.208

10. Sipos M, Gerszi D, Dalloul H, Banyai B, Sziva RE, Kollarics R, et al. Vitamin D Deficiency and Gender Alter Vasoconstrictor and Vasodilator Reactivity in Rat Carotid Artery. Int J Mol Sci. 2021;22(15).

IF: 6.208

11. Merkely P, Bakos M, Banyai B, Monori-Kiss A, Horvath EM, Bognar J, et al. Sex Differences in Exercise-Training-Related Functional and Morphological Adaptation of Rat Gracilis Muscle Arterioles. Front Physiol. 2021;12:685664.

IF: 4.755

12. Lajtai K, Tarszabo R, Banyai B, Peterffy B, Gerszi D, Ruisanchez E, et al. Effect of Vitamin D Status on Vascular Function of the Aorta in a Rat Model of PCOS. Oxid Med Cell Longev. 2021;2021:8865979.

IF: 7.310

13. Sipos M, Peterffy B, Sziva RE, Magyar P, Hadjadj L, Banyai B, et al. Vitamin D Deficiency Cause Gender Specific Alterations of Renal Arterial Function in a Rodent Model. Nutrients. 2021;13(2).

IF: 6.706

14. Lajtai K, Nagy CT, Tarszabo R, Benko R, Hadjadj L, Sziva RE, et al. Effects of Vitamin D Deficiency on Proliferation and Autophagy of Ovarian and Liver Tissues in a Rat Model of Polycystic Ovary Syndrome. Biomolecules. 2019;9(9).

IF: 4.082

15. Gerszi D, Penyige A, Mezei Z, Sarai-Szabo B, Benko R, Banyai B, et al. Evaluation of oxidative/nitrative stress and uterine artery pulsatility index in early pregnancy. Physiol Int. 2021;107(4):479-90.
IF: 2.090

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