

. 2025 Jul 1;163(1):71. doi: 10.1007/s00418-025-02397-y.

Distribution of endocannabinoid system receptors in the equine hoof: dysregulation as a potential therapeutic target for laminitis

Rodrigo Zamith Cunha ^{1 2}, Francesca Gobbo ³, Maria Morini ³, Augusta Zannoni ³, Carlo Mainardi ³, Lorenzo D'arpe ³, Alessandro Gramenzi ⁴, Roberto Chiocchetti ³

Affiliations Expand

PMID: 40593311

DOI: [10.1007/s00418-025-02397-y](https://doi.org/10.1007/s00418-025-02397-y)

Abstract

A growing body of evidence indicates that the endocannabinoid system (ECS) is essential for controlling the homeostasis of the skin and that the ECS is modified in the presence of skin disease. It is plausible to expect that the lamellar junction of the hoof expresses cannabinoid receptors and that their expression could be affected by lamellar disease. The goal of this study was to characterise the cannabinoid receptor type 1 (CB1R) and type 2 (CB2R) and the G protein-coupled receptor 55 (GPR55) within the dermo-epidermal junction of the hooves of healthy and laminitic horses. The expression of the CB1R, CB2R, and GPR55 within the dermo-epidermal lamellar junction of six healthy and 12 laminitic hooves was studied using polymerase chain reaction (PCR) and immunofluorescence. Both the mRNA and protein expression of the CB1R,

CB2R, and GPR55 were found in the dermo-epidermal lamellar junction of horse hooves. The immunolabelling was expressed by the epithelial cells of the primary and secondary laminae of healthy hooves (CB2R > GPR55 > CB1R). The presence of CB1R, CB2R, and GPR55 immunoreactivity in the healthy laminar epithelial cells, coupled with increased protein expression in pathological epithelial cells, provided strong motivation for future investigation. These findings suggest that cannabinoid compounds which interact with these receptors may influence lamellar healing and mitigate inflammation in hoof diseases, particularly laminitis.

Keywords: Cannabis sp.; Cannabinoid receptors; Epidermal lamellae; Horse; Lamellae.

© 2025. The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature.

[PubMed Disclaimer](#)

Conflict of interest statement

Declarations. Conflict of interest: The authors have no relevant financial or non-financial interests to disclose. Ethics approval and consent to participate: According to Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 regarding the protection of animals used for scientific purposes, Italian legislation (D. Lgs. n. 26/2014) did not require any approval by competent authorities or ethics committees as this study did not influence any therapeutic decisions. Consent for publication: All the authors have approved the publication of this manuscript.

Similar articles

[CB1R, CB2R and TRPV1 expression and modulation in in vivo, animal glaucoma models: A systematic review.](#)

Gallo Afflitto G, Aiello F, Scuteri D, Bagetta G, Nucci C.

Biomed Pharmacother. 2022 Jun;150:112981. doi: 10.1016/j.biopha.2022.112981. Epub 2022 Apr 22.

PMID: 35468582

[Decreased expression of p63, a regulator of epidermal stem cells, in the chronic laminitic equine hoof.](#)

Carter RA, Engiles JB, Megee SO, Senoo M, Galantino-Homer HL.

Equine Vet J. 2011 Sep;43(5):543-51. doi: 10.1111/j.2042-3306.2010.00325.x.

Epub 2011 Mar 11.

PMID: 21496086

[Risk factors for equine laminitis: a systematic review with quality appraisal of published evidence.](#)

Wylie CE, Collins SN, Verheyen KL, Newton JR.

Vet J. 2012 Jul;193(1):58-66. doi: 10.1016/j.tvjl.2011.10.020. Epub 2011 Nov 21.

PMID: 22104504

[Frequency of equine laminitis: a systematic review with quality appraisal of published evidence.](#)

Wylie CE, Collins SN, Verheyen KL, Richard Newton J.

Vet J. 2011 Sep;189(3):248-56. doi: 10.1016/j.tvjl.2011.04.014. Epub 2011 Jun 12.

PMID: 21665498

[Home treatment for mental health problems: a systematic review.](#)

Burns T, Knapp M, Catty J, Healey A, Henderson J, Watt H, Wright C.

Health Technol Assess. 2001;5(15):1-139. doi: 10.3310/hta5150.

PMID: 11532236

[See all similar articles](#)

References

Andradas C, C MM, P-G E, G M, S C (2013) The role of GPR55 in cancer. In: Actions at non-CB1/CB2 cannabinoid receptors. pp 115–133

Atalay S, Jarocka-Karpowicz I, Skrzydlewska E (2019) Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants* 9:21.

<https://doi.org/10.3390/antiox9010021> - DOI - PubMed - PMC

Baskerville CL, Chockalingham S, Harris PA, Bailey SR (2018) The effect of insulin on equine lamellar basal epithelial cells mediated by the insulin-like growth factor-1 receptor. *PeerJ* 6:e5945.

<https://doi.org/10.7717/peerj.5945> - DOI - PubMed - PMC

Belknap JK, Giguere S, Pettigrew A et al (2007) Lamellar pro-inflammatory cytokine expression patterns in laminitis at the developmental stage and at the onset of lameness: innate vs. adaptive immune response. *Equine Vet J* 39:42–47. <https://doi.org/10.2746/042516407X155406> - DOI -

PubMed

Blázquez C, Carracedo A, Barrado L et al (2006) Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB J* 20:2633–2635.

<https://doi.org/10.1096/fj.06-6638fje> - DOI - PubMed

Burns TA, Watts MR, Weber PS et al (2013) Distribution of insulin receptor and insulin-like growth factor-1 receptor in the digital laminae of mixed-breed ponies: an immunohistochemical study. *Equine Vet J*

45:326–332. <https://doi.org/10.1111/j.2042-3306.2012.00631.x> - DOI -

PubMed

Cassimeris L, Armstrong C, Burger QC et al (2021) Continuous digital hypothermia reduces expression of keratin 17 and 1L–17A inflammatory pathway mediators in equine laminitis induced by hyperinsulinemia. *Vet Immunol Immunopathol* 241:110326.

<https://doi.org/10.1016/j.vetimm.2021.110326> - DOI - PubMed

Cheng Z, Teo G, Krueger S et al (2016) Differential dynamics of the mammalian mRNA and protein expression response to misfolding stress. *Mol Syst Biol.* <https://doi.org/10.15252/msb.20156423> - DOI - PubMed -

PMC

PMC

Chiocchetti R, Galiazzo G, Tagliavia C et al (2019) Cellular distribution of canonical and putative cannabinoid receptors in canine cervical dorsal root ganglia. *Front Vet Sci* 6:313.

<https://doi.org/10.3389/fvets.2019.00313> - DOI - PubMed - PMC

Chiocchetti R, Rinnovati R, Tagliavia C et al (2021) Localisation of cannabinoid and cannabinoid-related receptors in the equine dorsal root ganglia. *Equine Vet J* 53:549–557. <https://doi.org/10.1111/evj.13305> - DOI - PubMed

Chiocchetti R, De Silva M, Aspidi F et al (2022) Distribution of cannabinoid receptors in keratinocytes of healthy dogs and dogs with atopic dermatitis. *Front Vet Sci*. <https://doi.org/10.3389/fvets.2022.915896> - DOI - PubMed - PMC

Czifra G, Szöllősi AG, Tóth BI et al (2012) Endocannabinoids regulate growth and survival of human eccrine sweat gland-derived epithelial cells. *J Investig Dermatol* 132:1967–1976. <https://doi.org/10.1038/jid.2012.118> - DOI - PubMed

Douglas JE, Thomason JJ (2000) Shape, orientation and spacing of the primary epidermal laminae in the hooves of neonatal and adult horses (*Equus caballus*). *Cells Tissues Organs* 166:304–318. <https://doi.org/10.1159/000016744> - DOI - PubMed

Elliott J, Bailey SR (2023) A review of cellular and molecular mechanisms in endocrinopathic, sepsis-related and supporting limb equine laminitis. *Equine Vet J* 55:350–375. <https://doi.org/10.1111/evj.13933> - DOI - PubMed

Ellis KL, Contino EK (2021) Treatment using cannabidiol in a horse with mechanical allodynia. *Equine Vet Educ*. <https://doi.org/10.1111/eve.13168> - DOI

Fails AD (2020) Functional anatomy of the equine musculoskeletal system. In: Adams and Stashak's lameness in horses. pp 1–65

Gabrielsson L, Mattsson S, Fowler CJ (2016) Palmitoylethanolamide for the treatment of pain: pharmacokinetics, safety and efficacy. *Br J Clin Pharmacol* 82:932–942. <https://doi.org/10.1111/bcp.13020> - DOI - PubMed - PMC

Galiazzo G, Giancola F, Stanzani A et al (2018) Localization of cannabinoid receptors CB1, CB2, GPR55, and PPAR α in the canine gastrointestinal tract. *Histochem Cell Biol* 150:187–205.

<https://doi.org/10.1007/s00418-018-1684-7> - DOI - PubMed

Galiazzo G, Tagliavia C, Giancola F et al (2021) Localisation of cannabinoid and cannabinoid-related receptors in the horse ileum. *J Equine Vet Sci* 104:103688. <https://doi.org/10.1016/j.jevs.2021.103688> - DOI - PubMed

Galiazzo G, De Silva M, Giancola F et al (2022) Cellular distribution of cannabinoid-related receptors TRPV1, PPAR-gamma, GPR55 and GPR3 in the equine cervical dorsal root ganglia. *Equine Vet J* 54:788–798.

<https://doi.org/10.1111/evj.13499> - DOI

Hargrove JL, Schmidt FH (1989) The role of mRNA and protein stability in gene expression. *FASEB J* 3:2360–2370.

<https://doi.org/10.1096/fasebj.3.12.2676679> - DOI - PubMed

Harvey BS, Nicotra LL, Vu M, Smid SD (2013) Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. *Cytokine* 63:209–217. <https://doi.org/10.1016/j.cyto.2013.04.032> - DOI - PubMed

Henstridge CM, Balenga NAB, Kargl J et al (2011) Minireview: recent developments in the physiology and pathology of the lysophosphatidylinositol-sensitive receptor GPR55. *Mol Endocrinol* 25:1835–1848. <https://doi.org/10.1210/me.2011-1197> - DOI - PubMed - PMC

Hill JD, Zuluaga-Ramirez V, Gajghate S et al (2018) Activation of GPR55 increases neural stem cell proliferation and promotes early adult

hippocampal neurogenesis. *Br J Pharmacol* 175:3407–3421.

<https://doi.org/10.1111/bph.14387> - DOI - PubMed - PMC

Iannotti FA, Di Marzo V, Petrosino S (2016) Endocannabinoids and endocannabinoid-related mediators: targets, metabolism and role in neurological disorders. *Prog Lipid Res* 62:107–128.

<https://doi.org/10.1016/j.plipres.2016.02.002> - DOI - PubMed

Kawasako K, Higashi T, Nakaji Y et al (2009) Histologic evaluation of the diversity of epidermal laminae in hooves of horses without clinical signs of laminitis. *Am J Vet Res* 70:186–193.

<https://doi.org/10.2460/ajvr.70.2.186> - DOI - PubMed

Kozubek M, Matula P (2000) An efficient algorithm for measurement and correction of chromatic aberrations in fluorescence microscopy. *J Microsc* 200:206–217. <https://doi.org/10.1046/j.1365-2818.2000.00754.x> - DOI - PubMed

Kupczyk P, Reich A, Szepietowski JC (2009) Cannabinoid system in the skin—a possible target for future therapies in dermatology. *Exp Dermatol* 18:669–679. <https://doi.org/10.1111/j.1600-0625.2009.00923.x> - DOI - PubMed

Kupczyk P, Rykala M, Serek P et al (2022) The cannabinoid receptors system in horses: tissue distribution and cellular identification in skin. *J Vet Intern Med* 36:1508–1524. <https://doi.org/10.1111/jvim.16467> - DOI - PubMed - PMC

Leise B (2018) The role of neutrophils in equine laminitis. *Cell Tissue Res* 371:541–550. <https://doi.org/10.1007/s00441-018-2788-z> - DOI - PubMed

Ligresti A, De Petrocellis L, Di Marzo V (2016) From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiol Rev* 96:1593–1659. <https://doi.org/10.1152/physrev.00002.2016> - DOI - PubMed

Liu B, Song S, Jones PM, Persaud SJ (2015) GPR55: from orphan to metabolic regulator? *Pharmacol Ther* 145:35–42.

<https://doi.org/10.1016/j.pharmthera.2014.06.007> - DOI - PubMed

Liu Y, Beyer A, Aebersold R (2016) On the dependency of cellular protein levels on mRNA abundance. *Cell* 165:535–550.

<https://doi.org/10.1016/j.cell.2016.03.014> - DOI - PubMed

Liu C, Li H, Xu F et al (2021) Cannabidiol protects human skin keratinocytes from hydrogen-peroxide-induced oxidative stress via modulation of the caspase-1–IL-1 β axis. *J Nat Prod* 84:1563–1572.

<https://doi.org/10.1021/acs.jnatprod.1c00083> - DOI - PubMed

Montecucco F, Di Marzo V (2012) At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. *Trends Pharmacol Sci* 33:331–340.

<https://doi.org/10.1016/j.tips.2012.03.002> - DOI - PubMed

Morales P, Hurst DP, Reggio PH (2017) Molecular targets of the phytocannabinoids: a complex picture. *Prog Chem Org Nat Prod* 103:103–131

Petrosino S, Di Marzo V (2017) The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. *Br J Pharmacol* 174:1349–1365.

<https://doi.org/10.1111/bph.13580> - DOI - PubMed

Piñeiro R, Maffucci T, Falasca M (2011) The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation.

Oncogene 30:142–152. <https://doi.org/10.1038/onc.2010.417> - DOI - PubMed

Polidoro G, Galiazzo G, Giancola F et al (2021) Expression of cannabinoid and cannabinoid-related receptors in the oral mucosa of healthy cats and cats with chronic gingivostomatitis. *J Feline Med Surg* 23:679–691.

<https://doi.org/10.1177/1098612X20970510> - DOI - PubMed

Pollitt CC (1996) Basement membrane pathology: a feature of acute equine laminitis. *Equine Vet J* 28:38–46.

<https://doi.org/10.1111/j.2042-3306.1996.tb01588.x> - DOI - PubMed

Pollitt CC (2010) The anatomy and physiology of the suspensory apparatus of the distal phalanx. *Vet Clin N Am Equine Pract* 26:29–49.

<https://doi.org/10.1016/j.cveq.2010.01.005> - DOI

Pryimak N, Zaiachuk M, Kovalchuk O, Kovalchuk I (2021) The potential use of cannabis in tissue fibrosis. *Front Cell Dev Biol*.

<https://doi.org/10.3389/fcell.2021.715380> - DOI - PubMed - PMC

Romero-Zerbo SY, Rafacho A, Díaz-Arteaga A et al (2011) A role for the putative cannabinoid receptor GPR55 in the islets of Langerhans. *J Endocrinol* 211:177–185.

<https://doi.org/10.1530/JOE-11-0166> - DOI - PubMed

Scheau C, Badarau IA, Mihai L-G et al (2020) Cannabinoids in the pathophysiology of skin inflammation. *Molecules* 25:652.

<https://doi.org/10.3390/molecules25030652> - DOI - PubMed - PMC

Silver RJ (2019) The endocannabinoid system of animals. *Animals* 9:686.

<https://doi.org/10.3390/ani9090686> - DOI - PubMed - PMC

Simcocks AC, O’Keefe L, Jenkin KA et al (2014) A potential role for GPR55 in the regulation of energy homeostasis. *Drug Discov Today*

19:1145–1151. <https://doi.org/10.1016/j.drudis.2013.12.005> - DOI - PubMed

Sugawara K, Zákány N, Tiede S et al (2021) Human epithelial stem cell survival within their niche requires “tonic” cannabinoid receptor

1-signalling—lessons from the hair follicle. *Exp Dermatol* 30:479–493.

<https://doi.org/10.1111/exd.14294> - DOI - PubMed

Tam J, Liu J, Mukhopadhyay B et al (2011) Endocannabinoids in liver disease. *Hepatology* 53:346–355. <https://doi.org/10.1002/hep.24077> -

DOI - PubMed

Tudurí E, López M, Diéguez C et al (2017) GPR55 and the regulation of glucose homeostasis. *Int J Biochem Cell Biol* 88:204–207.

<https://doi.org/10.1016/j.biocel.2017.04.010> - DOI - PubMed

Van Eps AW, Pollitt CC (2004) Equine laminitis: cryotherapy reduces the severity of the acute lesion. *Equine Vet J* 36:255–260.

<https://doi.org/10.2746/0425164044877107> - DOI - PubMed

Wang L-L, Zhao R, Li J-Y et al (2016) Pharmacological activation of cannabinoid 2 receptor attenuates inflammation, fibrogenesis, and promotes re-epithelialization during skin wound healing. *Eur J Pharmacol* 786:128–136. <https://doi.org/10.1016/j.ejphar.2016.06.006> - DOI -

PubMed

Wilkinson JD, Williamson EM (2007) Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *J Dermatol Sci* 45:87–92. <https://doi.org/10.1016/j.jdermsci.2006.10.009> - DOI - PubMed

Williams MR, Holbrook TC, Maxwell L et al (2022) Pharmacokinetic evaluation of a cannabidiol supplement in horses. *J Equine Vet Sci* 110:103842. <https://doi.org/10.1016/j.jevs.2021.103842> - DOI - PubMed

Yocom AF, O'Fallon ES, Gustafson DL, Contino EK (2022)

Pharmacokinetics, safety, and synovial fluid concentrations of single- and multiple-dose oral administration of 1 and 3 mg/kg cannabidiol in horses.

J Equine Vet Sci 113:103933. <https://doi.org/10.1016/j.jevs.2022.103933> -

DOI - PubMed

Zamith Cunha R, Semprini A, Salamanca G et al (2023a) Expression of cannabinoid receptors in the trigeminal ganglion of the horse. *Int J Mol Sci* 24:15949. <https://doi.org/10.3390/ijms242115949> - DOI - PubMed - PMC

Zamith Cunha R, Semprini A, Salamanca G et al (2023b) Expression of cannabinoid receptors in the trigeminal ganglion of the horse. *Int J Mol Sci*. <https://doi.org/10.3390/ijms242115949> - DOI - PubMed - PMC

Zamith Cunha R, Zannoni A, Salamanca G et al (2023c) Expression of cannabinoid (CB1 and CB2) and cannabinoid-related receptors (TRPV1, GPR55, and PPAR α) in the synovial membrane of the horse metacarpophalangeal joint. Front Vet Sci.

<https://doi.org/10.3389/fvets.2023.1045030> - DOI - PubMed - PMC

Zannoni A, Bombardi C, Dondi F et al (2014) Proteinase-activated receptor 2 expression in the intestinal tract of the horse. Res Vet Sci 96:464–471.

<https://doi.org/10.1016/j.rvsc.2014.03.006> - DOI - PubMed