# Canine Atopic Dermatitis: Skin Barrier Dysfunction and Topical Therapy

Canine atopic dermatitis (CAD) is a common, yet challenging chronic, inflammatory skin condition and its complex, multifactorial aetiology means a polymodal therapeutic approach can hold the key to successful long-term management. Conventional treatment choices often involve steroids and/or immunomodulatory drugs, which are known to have deleterious side-effects or may be contraindicated. In humans, extensive research has implicated the role of skin barrier dysfunction in the pathogenesis of the disease, hence topical therapy is the mainstay of treatment in human atopic dermatitis (AD) with approximately 75-80% of patients attaining safe and effective relief with topical methods alone.[1] Given pathogenetic similarities between dogs and humans, the use of topical therapies in CAD management is garnering greater attention within the veterinary community. This article reviews the evidence for skin barrier dysfunction in CAD and summarises the current evidence for the use of topical therapies in the management of the condition.

## The Epidermal Skin Barrier

The epidermis is the dynamic, self-restoring outermost layer of skin; acting as a protective, waterproof barrier to limit trans-epidermal water loss (TEWL) and percutaneous penetration of exogenous substances. The predominant epidermal cell type is the keratinocyte, originating in the germinal stratum basale then migrating through several more strata before reaching their terminal position in the stratum corneum (SC). During this journey keratinocytes evolve into tough, flattened corneocytes via the accumulation of keratin filaments, tightly-bound together by the protein filaggrin.[2] This process, known as keratinisation (or cornification), produces corneocytes which are perfectly adapted to perform their role as a primary barrier to the external world. Meanwhile, filaggrins undergo enzymatic degradation to produce natural moisturising factor (NMF), contributing to epidermal hydration and skin barrier function.[2] Keratinocyte cytoplasmic inclusions, called lamellar bodies (LB), exocytose specific lipids and proteins into the inter-corneocyte space to form the extracellular (EC) lipid matrix.[3] Contributing largely to the EC matrix is a family of bioactive lipids known as sphingolipids, with ceramides being the predominant type comprising approximately 50% of the total EC lipid content.[4] Fatty acids and cholesterols make up the remainder, and the resulting ratio is important for the maintenance of epidermal permeability barrier function.[3,4]

## Epidermal abnormalities

The role of epidermal abnormalities in skin barrier dysfunction has led to the outside-to-inside pathogenetic hypothesis of CAD (Figure 1): Alterations in epidermal proteins such as filaggrin, NMF and antimicrobial peptides (AMPs), along with abnormalities in the EC lipid composition and ultrastructure,[5,6] can allow increased transcutaneous penetration of allergens which incites and propagates the inflammatory cycle subsequently resulting in further barrier dysfunction, microbial colonisation and additional sensitisations (i.e. outside-inside-outside).[7,8,9] Humans and dogs share a remarkably comparable ceramide profile,[10] and due to striking similarities in the pathogenesis, immunological and histological changes, and clinical distribution of AD, canine models are commonly used for research into human disease.[5] Given the limitations commonly encountered in veterinary research, valuable and applicable inter-species comparisons are often made.

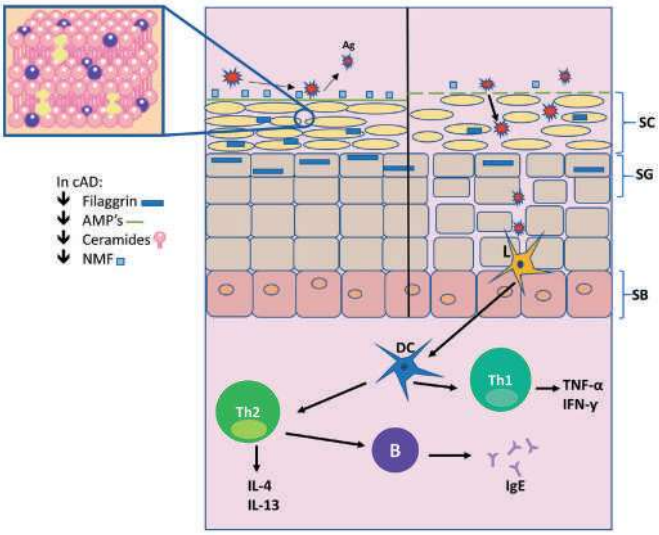


*Figure 1. The outside-to-inside pathogenesis of canine atopic dermatitis. Primary skin barrier abnormalities allow exogenous allergens (Ag) to penetrate the stratum corneum (SC) to interact with Langerhan cells (L) and stimulate the adaptive immune response (T-cell, B-cell). SG=Stratum Granulosum, SB=Stratum Basale, DC=Dendritic Cell, NMF-Natural Moisturising Factor, AMP- Antimicrobial Peptide.*

## Filaggrin

Filaggrin is an epidermal keratin-binding protein essential for effective formation of a functional cornified envelope, and following terminal enzymatic degradation creates hygroscopic NMF's,[11] important for maintaining skin hydration and barrier function.[2] Filaggrin reductions have been identified in atopic dogs through immunohistochemical staining,[12] although the relevance of this in relation to the severity of clinical signs remains unclear.[13] Filaggrin gene mutations (FLG) are well documented in atopic humans and are associated with abnormal epidermal differentiation and proliferation, and increased TEWL.[14,15] Approximately 20-30% of humans with AD are carriers of FLG mutations,[14] with a similar prevalence in atopic dogs of 22% reported by Chervet *et al.* However, abnormalities in filaggrin protein expression were seen in 83% of dogs,[15] mirroring findings in atopic humans where filaggrin deficiency was observed irrespective of FLG mutation.[16] Not only does this implicate filaggrin deficiency as a key factor in disease development, but indicates that factors other than genotype are instrumental in the filaggrin deficiency seen in AD.[14] Inflammation appears to be one of these factors, as multiple *in vitro* studies have demonstrated downregulation of filaggrin expression following cytokine stimulation.[17-19] *In vivo*, filaggrin and filaggrin-2 reductions have been identified in healthy and sensitised dogs respectively, following allergen challenge.[12,20] The relationship between CAD and FLG mutation remains unclear,[2] and conflicting results have been reported regarding filaggrin expression in atopic dogs, with upregulation,[20,21] no significant change,[22] or only breed-specific reductions observed.[23] Therefore, a complex interplay of primary genetic mutations, epigenetic changes

and environmental factors likely affect filaggrin expression and disease development.

**Antimicrobial Barrier**

Cutaneous AMPs have multifaceted roles in skin barrier homeostasis through regulation of the skin microbiome and by forming a crucial part of the innate immune system.[24] Decreased microbial diversity and increased relative abundance of certain commensal microbes have been identified in humans with AD.[25] Similarly in atopic dogs, superficial pyoderma lesions and skin exposed to allergen challenge demonstrated increases in Staphylococcus spp, particularly *S.pseudintermedius*, indicating an alteration to the cutaneous microbiota.[26,27] One possible mechanism for this alteration is the dysregulated expression and function of AMPs that have been identified in atopic dogs,[28] with specific decreases in anti-staphylococcal AMPs in the lesional skin of human AD patients also having been identified.[29] Impaired antimicrobial barrier function may explain the increased susceptibility of atopic patients to cutaneous microbial infections, and the isolation of bacteria containing ceramide-degrading enzymes from human AD patients could provide one mechanism whereby the antimicrobial and permeability barriers appear to show interdependence, with a failure in one initiating or exacerbating a failure in the other.[30,31] However, a better understanding of the genetic variability and expression of AMPs in normal and disease states in veterinary species is required as much for the current research is in humans.

**Extracellular lipids**

**1. Lipid metabolism and transport**

The cause of ceramide deficiencies seen in AD is subject to discussion, with many hypotheses pertaining to defects in epidermal lipid metabolism and impaired ceramide homeostasis.[32,33] In healthy individuals hydrolysis of sphingomyelin (SM) by the enzyme sphingomyelinase is important for the normal production of ceramides.[33] However, enhanced activity of SM deacylase has been identified in lesional and non-lesional skin of human AD patients and through increased competition for their common substrate (SM), ceramide production is reduced and an epidermal deficiency results.[32] Furthermore, atopic dogs have shown an epidermal accumulation of a ceramide precursor, glucocerebroside, due to decreased activity of β-glucocerebrosidase, an enzyme important for ceramide production and normal barrier function.[34] Lipid-secreting LB's found within maturing keratinocytes are integral to the formation of the waterproof epidermal barrier through extrusion of their contents into the EC space.[36] Although the formation, density and content of LB's appears normal,[35] atopic humans, mice and dogs have all demonstrated an increased number of retained LBs within developing corneocytes of the SC suggesting that some aspect of the exocytosis process is disrupted, resulting in abnormal and decreased lipid secretion.[6,35,36]

**2. Lipid organisation**

A healthy EC lipid matrix is a densely-packed and highly-ordered three-dimensional structure of lipid lamellae.[37] Changes in the lamellar organisation has been identified in atopic humans and appears to be strongly associated with an abnormal ceramide composition, increased TEWL and even disease severity.[37,38] Whilst evidence in atopic dogs is less abundant, comparable changes in lipid composition and organisation have been observed.[10,39–41] Electron microscopy has revealed that at baseline, atopic dogs had focally severe abnormalities in lamellar organisation, with intercellular spaces containing atypical, heterogenous or absent lipid material in disorganised lamellae,[41,51] which was further impaired following allergen challenge.[6]

**3.Lipid quantity and composition**

The major epidermal lipids exist in approximately equimolar amounts and their presence in physiological levels, particularly regarding ceramides, is intrinsically linked to the maintenance of normal skin barrier function.[8,42–44] Lipid abnormalities in humans with AD have been well documented, with multiple studies attributing skin barrier dysfunction to altered ceramide profiles, changes in ceramide chain length, and deficiencies in protein-bound ceramides.[32,42,45] The normal canine ceramide profile closely resembles that found in humans,[10] and similar ceramide reductions in both lesional and non-lesional skin of atopic dogs have been seen.[39,43,46] An association with increased TEWL has been observed suggesting such lipid abnormalities result in significant disruption to skin barrier function.[44–46] Stahl *et al* demonstrated that inflammation can further exacerbate ceramide deficiencies in sensitised dogs even in sites distant to the allergic insult. Interestingly, normalisation of ceramide levels was seen once the insult was removed, implying that skin barrier integrity is at least partially impacted by inflammation.[47] From a clinical perspective, this is consistent with the self-perpetuating and escalating nature of AD, supporting the importance of therapies which not only control inflammation, but also aid skin barrier repair. Hence, topical therapies tend to favour a sphingolipid or ceramide-dominant formulation with the aim to correct such deficiencies.[48–55]

**The Evolution of Topical Therapy**

Following this paradigm shift in our understanding of the pathogenesis of AD towards skin barrier dysfunction, employing an 'outside-in' approach could be as revolutionary in CAD as it has been in human AD; topical therapy is considered a first-line treatment for atopic paediatric patients,[56] and emollients are a mainstay of treatment in atopic adults.[57] Topical therapies are regularly employed in the management of dermatological conditions, however their action has traditionally been to soothe and moisturise the skin, remove debris and grease, or treat antimicrobial infections.[58] Sphingolipid-based therapies are one of the most recent advances in the topical management of AD, and therefore form the focus of the following sections which review topical sphingolipids and their potential to reinforce the epidermal barrier and ameliorate clinical signs associated with AD.

**Effects on visual scores and clinical signs**

The updated 2015 International Committee on Allergic Diseases of Animals (ICADA) guidelines on the treatment of CAD recognises the place of lipid-based topical formulations due to their success in humans, although acknowledge that further high-quality research is required for CAD.[59] Since 2015, further studies have demonstrated promising results; Marsella et al studied a group of house dust mite (HDM) sensitised atopic beagles experiencing bi-weekly allergen exposure and concurrent treatment with a topical sphingolipid and hyaluronic acid formulation as a monotherapy for 8 weeks. There was a significant reduction in Canine Atopic Dermatitis Extent and Severity Index (CADESI) score after 1 week (Figure 2A) and Pruritis Visual Analogue Score (PVAS) after 8 weeks (Figure 2B) in treated animals compared to the control group,[50] confirming findings from earlier research using topical ceramide formulations.[49–51] Other studies have failed to report significant differences in similar clinical parameters,[60,61] however it is difficult to directly compare these studies due to inconsistencies in the lipid formulation, study design and extraneous variables. In humans, the success of ceramide-based products in AD management has been widely reported, with evidence supporting improvements in clinical signs, sleep habits, pruritis, quality of life, severity of lesions and TEWL.[53–55,62]

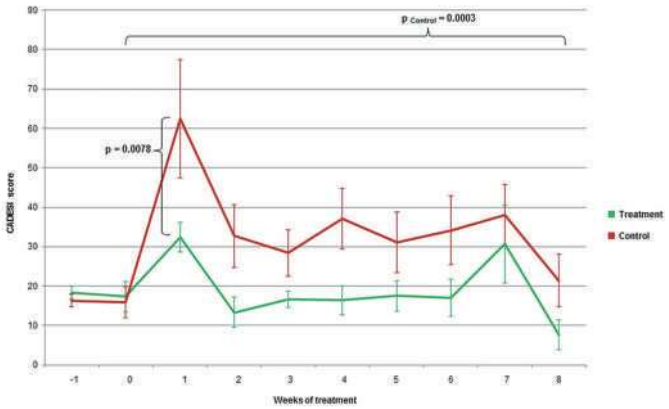


*Figure 2a: CADESI scores in treatment vs control group (Image from Marsella et al. 2020[48] under license CC-BY 4.0)*

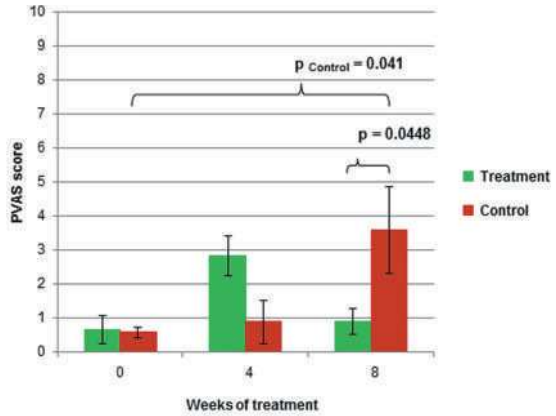


*Figure 2b: PVAS scores in treatment vs control group (Image from Marsella et al. 2020[48] under license CC-BY 4.0)*

### Drug-sparing effects in humans

The drug-sparing effect of topical therapies in humans with AD has been thoroughly established,[63,64] although the evidence base is smaller for more recently evolved topical sphingolipid therapies. However, comparative studies have demonstrated the efficacy of ceramide-dominant formulations when evaluated against pharmaceutical agents, with one product achieving an equivalent efficacy to fluticasone (a mid-potency topical steroid) as a sole therapy in reducing disease severity and pruritis in children with moderate to severe AD.[54] Another showed equal efficacy to pimecrolimus, a calcineurin inhibitor commonly used to manage human AD.[65] Such drugs have been associated with negative effects on skin barrier function through the reduction of numerous ceramide classes, LB secretion, antimicrobial function, and increased TEWL.[66,67] Although their potent immunomodulatory action addresses increased inflammatory cycling, prolonged use can contribute to barrier dysfunction.[66,67] Concurrent application of a ceramide-containing lipid mixture can mitigate these adverse effects by repairing barrier permeability and the interdependent anti-microbial barrier.[31,66] Although this has not yet been studied in dogs, these findings indicate a potential role for topical sphingolipids in facilitating an immunomodulatory drug-sparing effect, in addition to negating the adverse effects of these topical drug classes on both the permeability and antimicrobial skin barrier.

### Effects on extracellular lipids

The topical application of sphingolipids has been associated with a multifactorial effect on EC lipids: Increased lipid biosynthesis from keratinocytes, intercellular ceramide concentrations, LB extrusion and an improvement in ultrastructural organisation of lipid lamellae (Figure 3) following the application of ceramide-based formulations has been reported.[51,52,68] More recently, research has shown that the type of sphingolipid within a formulation affects its ability to alter epidermal lipid properties, with sphingomyelin(SM) rich lipid extracts appearing to exert greater beneficial effects. SM-rich lipid extracts applied to an *in vitro* canine skin model significantly increased total ceramide levels, with the most SM-rich extract (SPE-1) significantly increasing 44/99 ceramide metabolites,(Figure 4) whilst electron microscopy revealed increased intercellular lipids and LB's compared to pre-treatment levels.[69] Interestingly, no significant increases were seen in ceramide metabolites with the ceramide-rich extract, suggesting SM can support enhanced endogenous *de novo* ceramide production. Bearing in mind the positive results demonstrated *in vivo*,[48] SM-rich products could be a potentially exciting advancement in skin barrier repair for CAD, with the role of hyaluronic acid warranting further investigation.

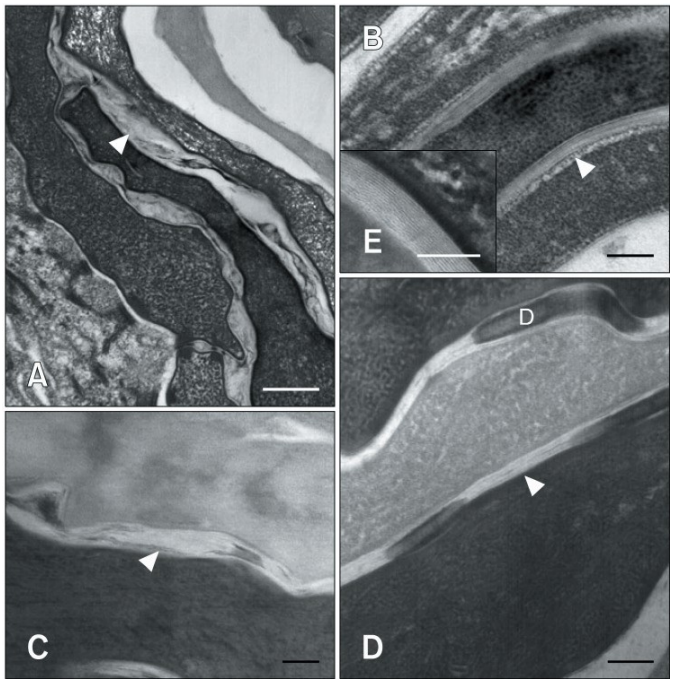


*Figure 3. A, C: Atopic dogs demonstrate reduced inter-corneocyte spaces filled with highly disorganised lipid lamellae. B, D: Following treatment, increased inter-corneocyte spaces filled with more organized lipid lamellae and an almost normal lipid bilayer. (E) Magnified field from Fig. 3B (Image from Jung et al[51] under license CC BY-NC 3.0)*

### Anti-inflammatory properties of sphingolipids

Sphingolipids have many biological functions in cell signalling and intracellular messaging,[4] and when applied exogenously may have anti-inflammatory effects: A significant inhibition in the secretion of the pro-inflammatory cytokine PGE2 was demonstrated following application of SM in various concentrations to stimulated canine keratinocytes *in vitro* (Figure 5).[70] PGE2 has been detected in biologically-active levels in human AD skin,[71] whilst both lesional and non-lesional skin in atopic dogs have shown increased expression of the enzyme prostaglandin E synthase 1, important for PGE2 synthesis.[72] Taken together these findings indicate reduction of PGE2 as a therapeutic goal in AD. Further *in vitro* studies have demonstrated similar anti-inflammatory effects through the inhibition of nitric oxide (NO) production, cyclooxygenase-2 (COX-2) and tumour necrosis factor- α (TNF-α) expression following treatment with a ceramide mixture,[73] even following application of a bacterial lipopolysaccharide.[74] Given the evidence for the down-regulation of ceramide levels[47,75] and filaggrin expression,[16-18] along with ultrastructural alterations[6] following inflammatory stimuli, these results may hold major significance in the management of canine inflammatory skin disorders.

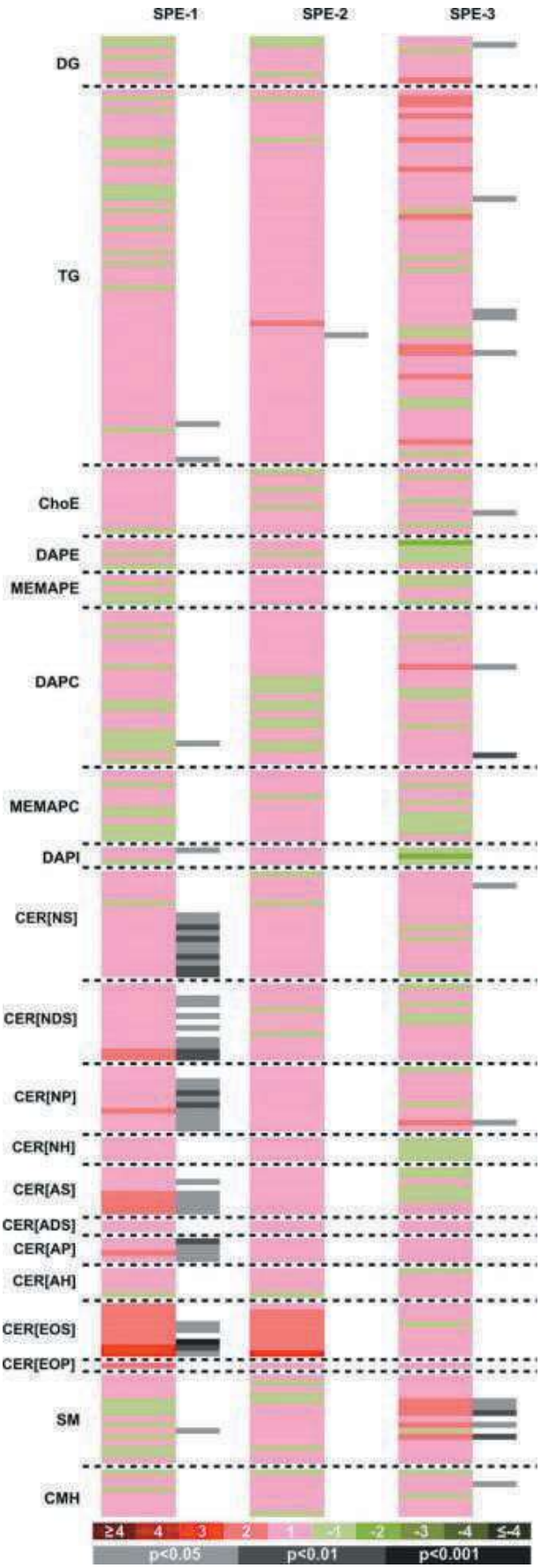


*Figure 4. Heat map showing lipid profile of different skin lipid metabolites obtained when comparing treatment groups (sphingolipid extracts: SPE-1, SPE-2 and SPE-3) with the control group. Red denotes increased level. Green denotes decreased level. (Image from Cerrato et al[69] under license CC BY-NC-ND 4.0)*

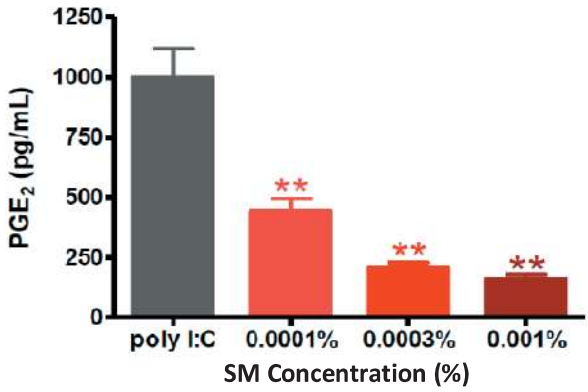


*Figure 5. (Image adapted from Cerrato et al[70])*

## Summary

Advancing the management of CAD has increasing importance due to the growing popularity of predisposed breeds and rising clinical prevalence. Only relatively recently has the concept of skin barrier dysfunction been recognised, with the outside-to-inside pathogenesis gaining greater traction within the wider veterinary community as a major therapeutic target. *In vivo* studies have demonstrated the potential for topical sphingolipid-based formulations to improve clinical signs and visual appearance of atopic lesions by reducing PVAS and CADESI scores. *In vitro* findings support these clinical observations by demonstrating the potential of sphingolipids to restore skin barrier permeability by increasing EC ceramide concentrations, LB extrusion and improving the ultrastructural organisation of lipid lamellae. The potential anti-inflammatory effects of topical sphingolipids indicate a further mechanism in mitigating the role of immunological and inflammatory mediators in the perpetuation of CAD, whether pre-existing or secondary to a fundamentally disrupted skin barrier. Whilst cautious extrapolation of data between species is prudent, it is difficult to ignore the striking similarities in pathogenesis, clinical distribution and immunological patterns seen in humans and dogs, and applicable comparisons between species can be valuable particularly given limitations within veterinary research. As such, topical sphingolipid formulations may be considered a safe, adjunctive therapy in the long-term, multimodal management of CAD.

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