



Phospholipids in sera of horses with summer eczema: Lipid analysis of the autoserum preparation used in therapy

R. E. HALLAMAA*^{†‡}, K. C. BATCHU[§] and T. TALLBERG[†]

[†]Institute for Bioimmunotherapy, Helsinki, Finland

[‡]Veterinary Clinic, Nummela, Finland

[§]Institute of Biomedicine, Department of Medical Biochemistry and Developmental Biology, University of Helsinki, Finland.

*Correspondence email: raija.hallamaa@elisanet.fi; Received: 29.09.12; Accepted: 29.06.13

Summary

Reasons for performing study: Equine summer eczema, also known as insect bite hypersensitivity, affects horses recurrently during summer months. The treatment of this allergic pruritus is difficult and therefore there is a need for efficacious treatments. Autoserum therapy, based on the use of autogenous serum that is specifically prepared for oral administration and given when the animal shows clinical signs has been introduced recently. Lipids are thought to be responsible for the effect of this therapy.

Objectives: The main aim of this study was to analyse the phospholipid content of autogenous serum preparations and to further assess whether these preparations have different lipid profiles depending on the clinical status of the horse. The hypothesis is that the major serum phospholipids typical of the horse are present in the autoserum preparation.

Study design: Descriptive controlled clinical study.

Methods: Sera were collected from 10 affected and 6 healthy horses, prepared in a similar fashion and the lipids contained in the resulting autoserum preparations were analysed by electrospray ionisation mass spectrometry.

Results: The major phospholipid classes detected were phosphatidylcholine, sphingomyelin, phosphatidic acid and traces of lysophosphatidylcholine. Horses with summer eczema had significantly abundant concentrations of phosphatidylcholine ($P = 0.042$) and sphingomyelin ($P = 0.0017$) in comparison with healthy horses, while the concentration of phosphatidic acid was significantly higher in healthy horses ($P = 0.0075$).

Conclusions: The autoserum preparation contains minute amounts of the main serum phospholipids in differing concentrations in healthy horses and horses with an allergic skin disease.

Keywords: horse; summer eczema; insect bite hypersensitivity; allergic dermatitis; autoserum therapy; phospholipids

Introduction

Equine summer eczema is the most common skin disease of the horse [1,2]. This allergic pruritus is associated with biting insects, especially species of *Culicoides* [1,3], and therefore is also known as insect bite hypersensitivity (IBH) or insect hypersensitivity [4,5]. The typical primary clinical sign is pruritus with secondary skin lesions, and horses are affected recurrently during the summer months when insects are active. So far, a clear hereditary influence on the sensitivity to the disease has not been found [6,7]. Immunoglobulin E (IgE) and reduced regulatory immune response of T cells have been linked to the pathogenesis [7–10], i.e. the overproduction of inflammatory agents combined with the dysfunction of regulatory mechanisms. Treatment is difficult and total isolation from biting insects is impossible to achieve in many settings.

Autoserum therapy for equine summer eczema has been advocated recently [11,12]. This therapy is based on the use of a horse's own serum that is specifically prepared: successively diluted and mechanically shaken with water and ethanol and finally absorbed into sugar granules used for oral medication [12]. The underlying hypothesis is that horses suffering from summer eczema overproduce lipids that are thereby released into their blood [11]. These lipid particles could form abnormal aggregate complexes that are incapable of interacting with their corresponding receptors on the plasma membrane, thus preventing cell signalling. During the autoserum preparation, these complexes are dissolved, and lipid molecules are again usable for biological reactions.

Lipids are insoluble in water and due to this hydrophobic nature they have an ability to form membranes [13,14]. Phospholipids are the major structural components of the plasma membrane where they are unequally distributed in its outer and inner leaflets. This lipid asymmetry is a vital element for transbilayer trafficking, and changes in the normal lipid asymmetry influence several cellular actions [15–18]. In addition, lipids are involved in a plethora of intra- and extracellular reactions including cell signalling, apoptosis and blood clotting [13–15,19]. The term lipidome

refers to the total cellular lipids that are specific to different cell types [13,14,20]. Mass spectrometric techniques have enabled quantitative analysis of a multitude of lipid species in mammalian cells [13,14,21] but the roles of many lipids are still unclear [14,18,21].

The objectives of the current study were to analyse by mass spectrometry the lipid content of the autoserum preparations made both from the sera of horses with summer eczema and from healthy controls and to determine if the preparations differ in their lipid profiles depending on the clinical status of the horses.

Materials and methods

Horses

Sera from 16 horses were collected for the analysis of autoserum preparations. Of these horses, 10 suffered from summer eczema (eczema group), while 6 were healthy horses (control group). The eczema group included 4 male and 6 female horses and their ages ranged from 2 to 12 years (mean $\pm$ s.d. 7.0 ± 3.4). Seven were Finnhorses and the others were a New Forest pony, a Shetland pony and an Icelandic horse. Horses had suffered from typical clinical signs for 2 months to 4 years (2.2 ± 1.2 years). Clinical signs were categorised as mild, moderate or severe depending on the size of skin lesions. Signs were classified as mild if pruritus was the only clinical sign, whereas pruritus with mild skin affections in the mane, tail and/or body were regarded as moderate signs. Horses with pruritus over large skin lesions were graded as having severe signs. Three of the horses had mild, 5 moderate and 2 horses suffered from severe signs. Horses had not been treated with glucocorticoids or antihistamines for at least 2 weeks before their sera for the preparation were collected. The samples for the study were collected at the same time the blood samples for their autoserum therapy were acquired.

TABLE 1: Phosphatidylcholine (PC) species detected in the autoserum preparations of healthy horses and horses with summer eczema (n = 16). The most abundant species are typed in bold

28:0	30:0	30:1	31:0	31:1	32:0	32:1	33:0	33:1	33:2
33:3	34:0	34:1	34:2	34:3	35:0	35:1	35:2	35:3	36:0
36:1	36:2	36:3	36:4	37:0	37:1	37:2	38:0	38:1	38:2
38:3	38:4	38:5	39:0	40:0	40:1	40:2	40:4	40:5	40:6

The control group comprised 6 healthy horses that were kept in the same stables as horses suffering from summer eczema. All the horses of this group were Finnhorses; there were 4 males and 2 females. Their ages ranged from 3 to 29 years (17.3 ± 8.7); horses aged ≥ 12 years were preferred, since it is unlikely that summer eczema will occur after this age in Finnhorses [22]. In the control horses, blood sampling was part of routine health monitoring programmes. These studies of the therapy have been approved by the Animal Care and Use Committee of the University of Helsinki. Owners' permission for inclusion in the study was obtained.

Timing and preparation of autoserum

Sera of the eczema group were collected when a horse had shown typical clinical signs for at least 2 weeks. Samples were also drawn from the control group at the same time. Serum was harvested after the erythrocytes had clotted without using a centrifuge to avoid destruction of serum lipids before harvesting. With a calibrated pipette, 0.065 ml of the serum was taken from the superficial layer, where serum lipids float. These lipid particles were then mechanically shaken with sterile water (1:100) in 2 serial dilutions in order to dissolve all the water-soluble molecules, accordingly 0.065 ml taken from the superficial layer of the previous dilution. Finally, 0.065 ml taken superficially of the latter solution was diluted and shaken (1:100) with 48% ethanol.

Lipid analysis of the autoserum preparation

The samples in ethanol were dried under a N_2 stream and the residue was reconstituted in 500 μ l chloroform/methanol (1:2) and stored at -20°C . From each sample, 50% was taken for a preliminary mass spectrometry analysis to ascertain the constitution of the lipids in it and unlabelled external standards were added to the remainder in order to quantify the amount of the lipids. After addition of aqueous NH_4OH (4%), the sample was infused at 6 μ l/min to a Micromass Quattro Micro triple-quadrupole mass spectrometer^a [23]. Specific scans in both positive and negative modes for the detection of individual phospholipid classes were performed including phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidic acid (PA), phosphatidylinositol and sphingomyelin (SM) based on their head groups, and species based on their acyl chain compositions. Spectra were acquired for 5 min over a mass range of m/z (mass-to-charge-ratio) 400–950 at a frequency of 4 scans/min. Lipid standards, di-22:1 PC, lysophosphatidylcholine (lysoPC) 20:0 and di-18:1 PA were each added to all the samples in order to quantify their concentrations. The spectra obtained were exported to Microsoft Excel^b and the detected lipids were quantified by using the LIMS software^c. The quantitative values were calculated by using peak intensities.

Laboratories

Two laboratories participated in the study. All autoserum preparations were made in one laboratory and all the lipid analyses were performed in the other. Researchers processing the samples were not aware of the horses' grouping.

Data analysis

Differences between phospholipid concentrations in the eczema and control groups were analysed by using the Mann–Whitney test due to nonparametric distribution of the lipid concentration data, according to the Shapiro–Wilk test. Spearman's correlation test was used to assess the relationship between phospholipid concentrations and clinical signs or the age of the horses. Analyses were performed using statistical software

StatsDirect^d and PASW Statistics 18.0 (SPSS)^e. Two-sided P values <0.05 were considered significant.

Results

Phospholipids are composed of a polar head group, a phosphate group, acyl chains and a glycerol backbone (glycerophospholipids) or a sphingosine backbone (sphingolipids) [13,19]. The polar head group defines the phospholipid class and based on their acyl chain composition (degree of unsaturation, chain length) phospholipids are divided into different molecular species. The simplified nomenclature of fatty acids is based on the number of carbon atoms and double bonds in unbranched acyl chains, i.e. PC 34:2 has 34 carbon atoms with 2 double bonds [13].

The most abundant species found in the positive scan mode by scanning for precursors of $m/z +184$ were that of PC species of 34:0, 34:1, 34:2, 36:0, 36:1 and 36:2 (Fig 1). In addition, PC 36:4 was present in very minor quantities in all the samples and was comparable across all of them. Many other PC head group species were also detected and are listed in Table 1. There were traces of SM species of 16:0, 18:0 and 21:0, and lysolipids (lysoPC 16:0, 18:0, 18:1 and 18:2) were also detected in all of the samples. Of these SM species 16:0 was the most abundant across all the samples. Among the negative charged lipids, only PA species of 32:0 and 34:1 were found (Fig 2). To confirm that the detected PA peak was indeed that of 32:0, further fragmentation was done, which resulted in the generation of a PA head group and a glycerol backbone. Specific head group scans were also carried out on the samples for the detection of other possible phospholipid head group species (phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and phosphatidylinositol), but none of them were found.

When the eczema group was compared with the control group, there were differences found in the profiles of PC, SM and PA species (Figs 3–5). The PC concentration was significantly higher in the eczema than in the control group ($P = 0.042$). Although SM was found only in minor amounts in the samples, there was a significant difference between the groups ($P = 0.0017$). The PA species were the only phospholipids that were found more abundantly in the control than in the eczema group ($P = 0.0075$). Traces of lysoPC were found in all samples, but were not significantly different between the groups.

Comparisons of phospholipid concentrations and severity of the clinical signs showed significant associations (Table 2). The larger the amount of PC or SM was, the more marked were the clinical signs. Conversely, PA had a significant negative relationship to severity of the disease.

One horse in the eczema group was an outlier. While the other horses in this group showed quite similar concentrations of PC and PA, this horse had markedly reverse profiles for both these phospholipids (Figs 3, 5). In the control group, no marked differences were found between the horses (Figs 3–5). In the eczema group, the mean $\pm$ s.d. age of the 5 youngest horses was 4.2 ± 1.3 years and of the 5 oldest horses 9.8 ± 2.2 . There was no significant association between the age of the horse and the phospholipid concentrations.

TABLE 2: Correlations (r) between phosphatidylcholine (PC), sphingomyelin (SM) and phosphatidic acid (PA) concentrations and clinical signs of the horses (n = 16)

	PC	SM	PA	Signs
PC, r	1.000	0.612*	-0.744**	0.792**
P		0.012	0.001	<0.001
SM, r	0.612*	1.000	-0.576*	0.822**
P	0.012		0.019	<0.001
PA, r	-0.744**	-0.576*	1.000	-0.801**
P	0.001	0.019		<0.001
Signs, r	0.792**	0.822**	-0.801**	1.000
P	<0.001	<0.001	<0.001	

*Correlation is significant at 0.05 level (two-sided). **Correlation is significant at 0.01 level (two-sided).

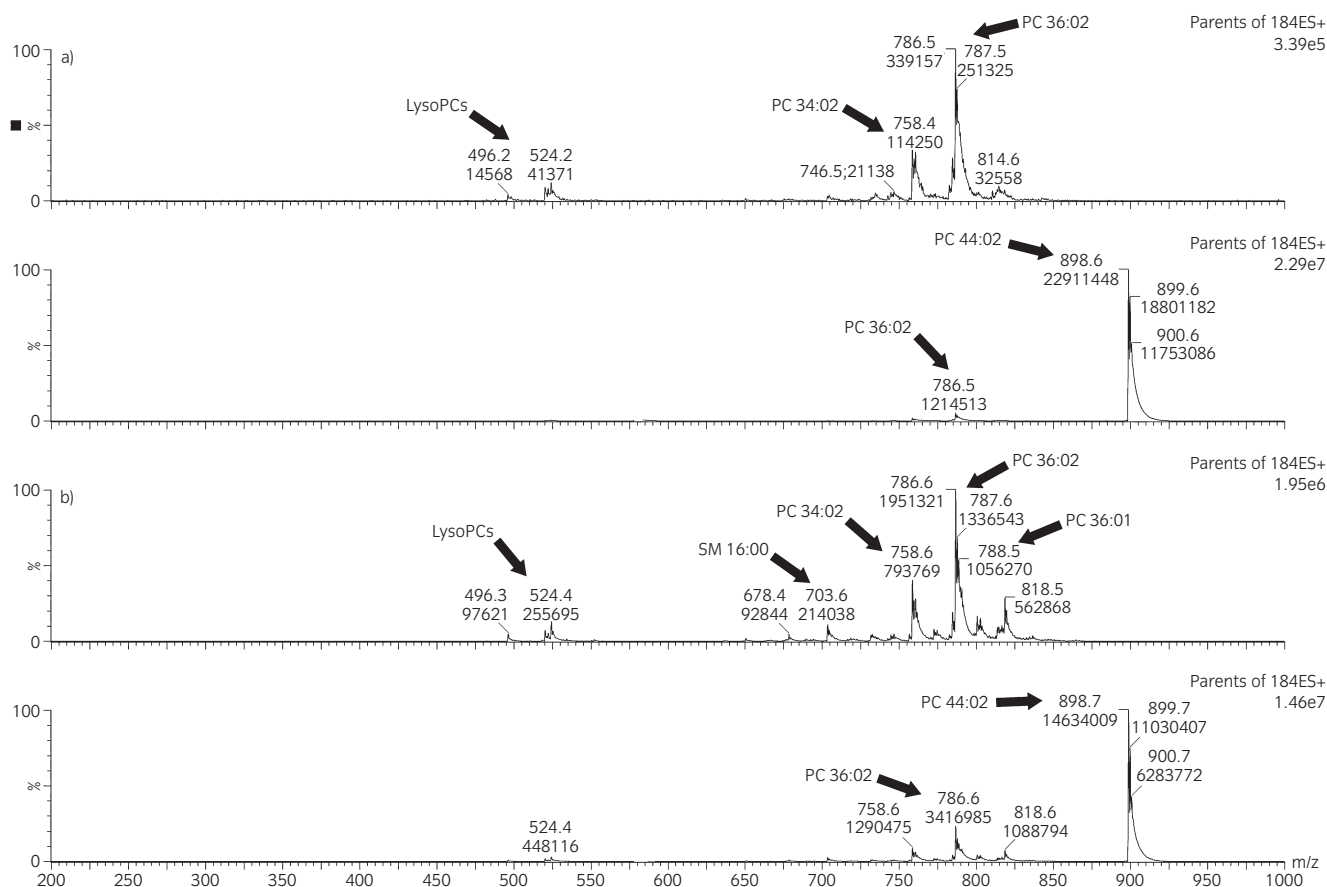


Fig 1: Electrospray ionisation mass spectrometry/mass spectrometry spectra obtained from precursors of m/z +184 scan mode. In (a), the top panel shows phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC) and sphingomyelin (SM) species detected in the serum sample of a horse belonging to the control group. The lower panel shows the spectra for the same sample with standards added to it. In (b), the top panel shows PC, lysoPC and SM species detected in the serum sample of a horse from the eczema group. The lower panel shows the spectra for the same sample with standards added to it.

Discussion

The current study was designed to analyse the lipid content of the autoserum preparations. The hypothesis proposed was that lipids in general are detectable in these serum preparations, despite serial dilutions. The study was based on the fact that lipids would be the main molecules that are able to survive following dilutions with water and finally with 48% ethanol. The sera were not analysed before the dilutions, since the purpose of the present work was to study components of the final dilution that was used in the treatment and could be expected to have a beneficial effect.

Phosphatidylcholine, SM and lysoPC are phospholipids commonly found in mammalian sera [24,25]. The current study showed that all the major serum phospholipids typical of the horse were detected in the preparations. The large amount of PA, especially in healthy horses, was a surprising finding. Phosphatidic acid is the precursor in lipid biosynthesis, a parent compound for other glycerophospholipids, and it is mainly situated in the inner leaflet of the plasma membrane [13]. Phosphatidic acid has not been demonstrated in the earlier studies on horses [24,25]. One explanation for the differences in our results when compared with a previous study [25] could be that the sera of their samples were harvested with a centrifuge, whereas in the current study centrifugation was not used. Although the erythrocyte sedimentation rate of the horse is rapid, our samples might still have a few blood cells, thus leading to detectable concentrations of PA. Similarly, the presence of PA in larger amounts in the control compared with the eczema samples may be due to the presence of more red blood cells in the control group, since the sedimentation rate is

generally slower in healthy animals than those with inflammation. It should also be noted that the samples of the present study were collected from the superficial layer of the sera, where the lightest and most hydrophobic molecules are likely to be concentrated. Therefore, the results of the present work are not strictly comparable with earlier studies.

There were significant differences between the horses with summer eczema and healthy horses in all of the main phospholipid classes (PC, SM and PA). It is unlikely that the alterations in the lipid profiles had resulted from variations in the diets of the animals [24–26]. Both control and patient groups were kept on pasture and thus nutritional conditions were similar. The differences between the results could have been due to miscellaneous working habits in the laboratories where the autoserum had been prepared or where the analysis of lipids had been done. In both laboratories, the same person had handled all the samples, except for one sample that had been prepared by another person. The result for this sample was similar to other horses in its group.

Both PC and SM were found more abundantly in the serum of the affected than in the healthy horses, which supports the hypothesis that horses with summer eczema excrete abnormally large amounts of lipid components into their blood [11]. Phosphatidylcholine and SM are typically found in the plasma membrane, in its outer leaflet [13,18,27] and they are amphipathic in nature, suggesting that they contain both hydrophobic and hydrophilic regions [13]. When amphipathic molecules are mixed with water molecules, the hydrophilic part favours water molecules, while the hydrophobic avoids them, leading to an aggregation of the lipid particles [13]. However, lipids are transported via membranes as monomers [18,21]. In horses with summer eczema, the large amounts of PC or SM could

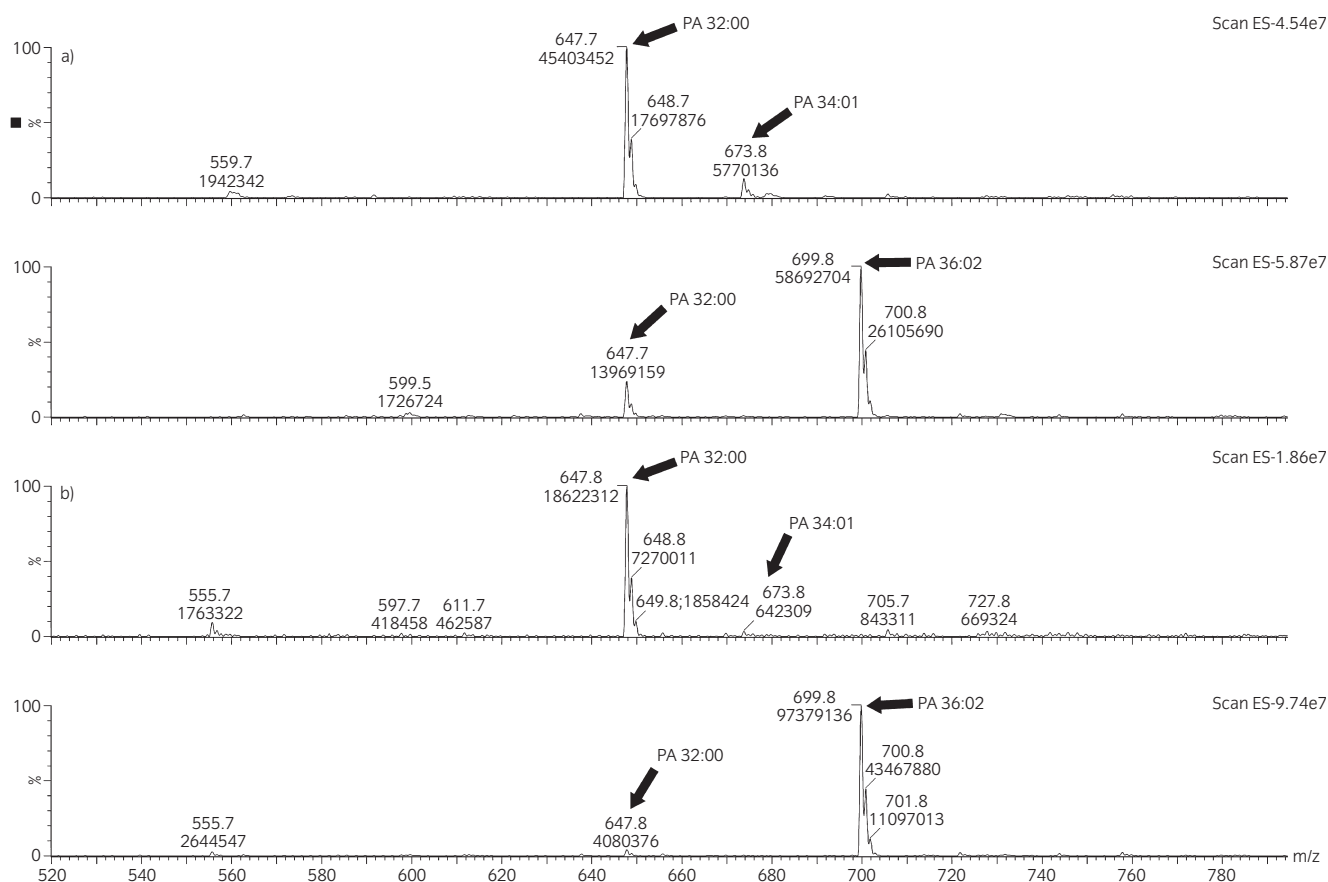


Fig 2: Electrospray ionisation mass spectrometry/mass spectrometry spectra obtained from the negative scan mode. In (a), the top panel shows only phosphatidic acid (PA) species detected in the serum sample of a horse belonging to the control group. The lower panel shows the spectra for the same sample with a standard added to it. In (b), the top panel shows only PA species detected in the serum sample of a horse from the eczema group. The lower panel shows the spectra for the same sample with a standard added to it.

predispose lipid molecules to spontaneously aggregate, preventing binding to their receptors on the plasma membrane and impeding distinct signalling pathways. During preparation these clusters may be segregated back to monomers. However, this explanation needs further specific

studies and the suggestion that lipids are responsible for the beneficial clinical effect of this autoserum therapy [11,12] remains to be proven.

Although both PC and SM were abundantly present in the sera of affected horses and their amount increased significantly according

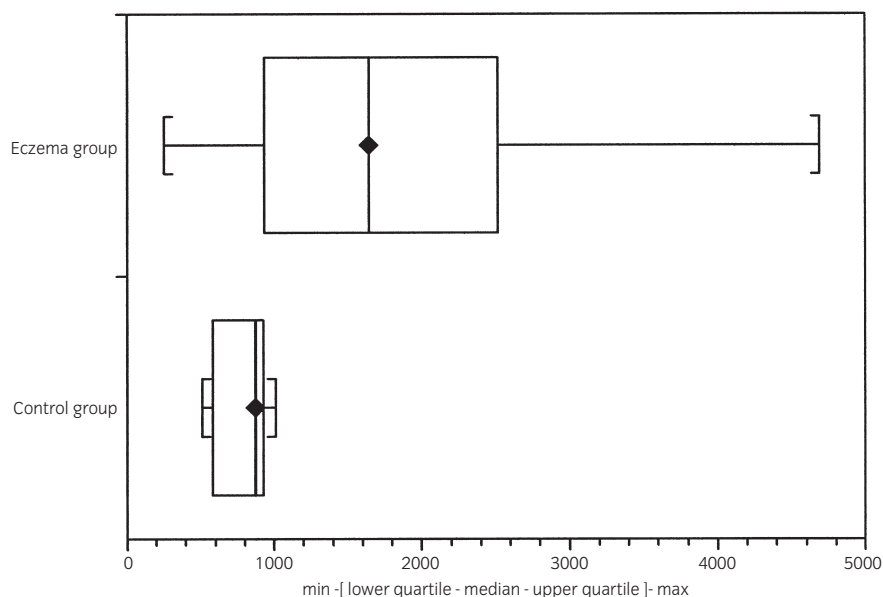


Fig 3: Phosphatidylcholine (PC) concentrations (pmol in total of 500 µl) of healthy control horses (n = 6) and horses with summer eczema (n = 10).

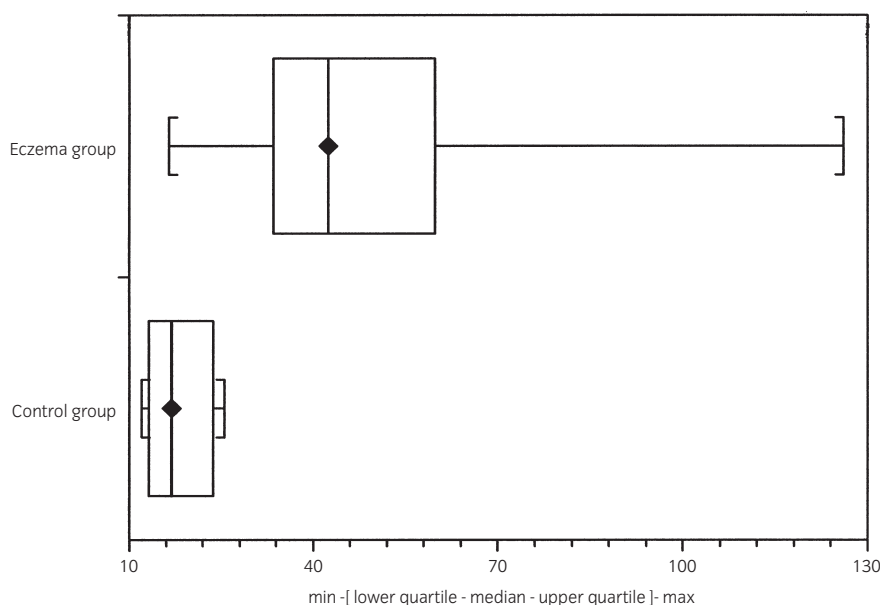


Fig 4: Sphingomyelin (SM) concentrations (pmol in total of 500 µl) of healthy control horses (n = 6) and horses with summer eczema (n = 10).

to severity of clinical signs, the individual roles of these phospholipids in the course of this allergic disease remains unspecified. The total amount of PC was larger, but SM might be the more relevant candidate that has an inductive or regulatory effect, since sphingolipids play essential roles in epidermal barrier metabolism and as messengers between extra- and intracellular spaces [13,18,28,29]. Sphingomyelin is a derivative and important source of ceramide that is the main component of epidermal barrier lipids [13,29]. In atopic dermatitis, changes in this skin barrier are suggested to be a crucial event in the pathogenesis of the disease [29]. Ceramide is actively synthesised after skin injuries and in chronic disorders the epidermal barrier needs constant repairing [29]. Sphingosine-1-phosphate (S1P), also generated of ceramide and SM, is a lipid mediator that participates in multiple physiological actions, especially in mast cells [28]. The engagement of the IgE receptor on mast cells leads to the production of S1P and its secretion into the extracellular space [28]. The S1P induces mast cell degranulation, but also controls systemic influences after mast cell activation, e.g. by preventing dendritic cell migration to the lymph nodes [28]. Patients with allergic dermatitis or

psoriasis show marked expressions of enzymes that degrade S1P, which may affect the persistence of the disease [28]. The larger amounts of SM in the eczema horses may indicate that SM has not been used normally in corresponding reactions or it has been excessively produced. Therefore, the significant increase of SM in the affected horses is an interesting finding, even though only small amounts were found in the analysis. Nevertheless, a possible metabolic action cannot be unambiguously linked to the total amount of the different lipids, since only minute amounts of lipid messengers are needed for signalling [18]. Consequently, further studies on a greater number of horses are necessary to evaluate the role of SM in equine summer eczema.

In conclusion, the present study has introduced a potential example of the dynamic roles of lipids in equine health and disease. Horses with an allergic skin disease have different concentration profiles of the main serum phospholipids when compared with healthy horses. The link between serum phospholipids, autoserum therapy and equine summer eczema requires further investigation.

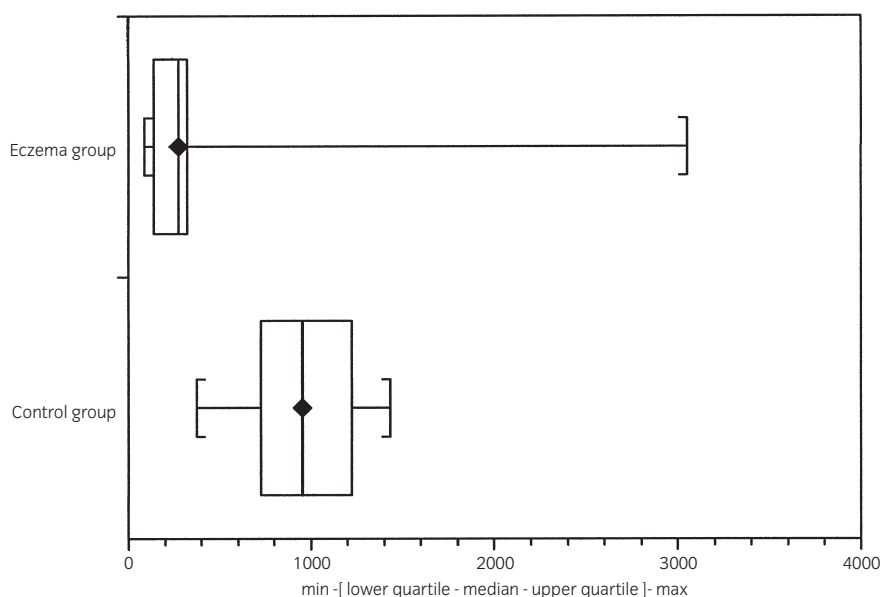


Fig 5: Phosphatidic acid (PA) concentrations (pmol in total of 500 µl) of healthy control horses (n = 6) and horses with summer eczema (n = 10).

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

This study was approved by the Animal Care and Use Committee of the University of Helsinki and the study was performed with owners' permission.

Source of funding

This study was supported by the Albert Lindsay von Julin Foundation.

Acknowledgements

We thank Dr Jan Dabek and Doc Marja Raekallio for their valuable advice and suggestions and Professors Pentti Somerharju and Outi Vainio for their cooperation with this study. We are grateful to Mrs Tarja Grundstrom and Mrs Leena Wathen for their excellent laboratory assistance.

Authorship

We state that all authors have contributed to this study. R.E. Hallamaa and T. Tallberg designed this study. R.E. Hallamaa collected the samples and wrote the main text of the manuscript and T. Tallberg made his suggestions and revisions to the final manuscript. K.C. Batchu performed the lipid analyses and made additions and revisions to that part of the text, and also checked the final manuscript.

Manufacturers' addresses

^aWaters, Milford, Massachusetts, USA.

^bMicrosoft Corporation, Redmond, Washington, USA.

^cUniversity of Helsinki, Helsinki, Finland.

^dStatsDirect Ltd., Sale, Cheshire, UK.

^eIBM Corporation, Armonk, New York, USA.

References

- Barbet, J. (1992) *Culicoides* hypersensitivity. In: *Current Therapy in Equine Medicine*, Ed: L. Mills, W. B. Saunders, Philadelphia. pp 693-696.
- Scott, D.W. and Miller, W.H. Jr (2003) Insect hypersensitivity. In: *Equine Dermatology*, Eds: R. Kersey and D. LeMelledo, Saunders, Elsevier Science, St Louis. pp 458-474.
- Halldorsdottir, S. and Larsen, H.J. (1989) Intradermal challenge of Icelandic horses with extracts of four species of the genus *Culicoides*. *Res. Vet. Sci.* **47**, 283-287.
- Kurotaki, T., Narayama, K., Oyamada, T., Yoshikawa, H. and Yoshikawa, T. (1994) Immunopathological study on equine insect hypersensitivity ('Kasen') in Japan. *J. Comp. Pathol.* **110**, 145-152.
- van Grevenhof, E.M., Ducro, B., Heuven, H.C.M. and Bijma, P. (2007) Identification of environmental factors affecting the prevalence of insect bite hypersensitivity in Shetland ponies and Friesian horses in the Netherlands. *Equine Vet. J.* **39**, 69-73.
- Björnsdottir, S., Sigvaldardottir, J., Broström, H., Langvad, B. and Sigurosson, A. (2006) Summer eczema in exported Icelandic horses: influence of environmental and genetic factors. *Acta Vet. Scand.* **48**, 3; doi: 10.1186/1751-0147-48-3.
- Schaffartzik, A., Hamza, E., Janda, J., Cramer, R., Marti, E. and Rhyner, C. (2012) Equine insect bite hypersensitivity: what do we know? *Vet. Immunol. Immunopathol.* **147**, 113-126.
- Wagner, B., Miller, W.H., Morgan, E.E., Hillegas, J.M., Erb, H.N., Leibold, W. and Antczak, D.F. (2006) IgE and IgG antibodies in skin allergy of the horse. *Vet. Res.* **37**, 813-825.
- Hamza, E., Steinbach, F. and Marti, E. (2011) CD4+CD25+ T cells expressing FoxP3 in Icelandic horses affected with insect bite hypersensitivity. *Vet. Immunol. Immunopathol.* **148**, 139-144.
- Heimann, M., Janda, J., Sigurdardottir, O.G., Svansson, V., Klukowska, J., von Tschärner, C., Doherr, M., Broström, H., Andersson, L.S., Einarsson, S., Marti, E. and Torsteinsdottir, S. (2011) Skin-infiltrating T cells and cytokine expression in Icelandic horses affected with insect bite hypersensitivity: a possible role for regulatory T cells. *Vet. Immunol. Immunopathol.* **140**, 63-74.
- Hallamaa, R.E., Lepistö, R.L. and Tallberg, T. (2001) Treatment of equine summer eczema with an autogenous serum preparation, possibly effected by inductive lipid signals. *Dtsch. Zschr. Onkol.* **33**, 57-62.
- Hallamaa, R.E. (2010) Autoserum preparation in the treatment of equine summer eczema: findings over 12 years. *Equine Vet. Educ.* **22**, 610-615.
- Nelson, D.L. and Cox, M.M. (2008) Lipids, biological membranes and transport. In: *Principles of Biochemistry*, 5th edn., Ed: K. Ahr, W.H. Freeman, New York. pp 343-380.
- van Meer, G. (2005) Cellular lipidomics. *EMBO J.* **24**, 3159-3165.
- Fadeel, B. and Xue, D. (2009) The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **44**, 264-277.
- Silveira E Souza, A.M., Mazucato, V.M., Jamur, M.C. and Oliver, C. (2011) Lipid rafts in mast cell biology. *J. Lipids* **2011**, 752906; doi: 10.1155/2011/752906.
- van Meer, G. (2011) Dynamic transbilayer lipid asymmetry. *Cold Spring Harb. Perspect. Biol.* **3**, a004671.
- van Meer, G. and de Kroon, A.I.P.M. (2011) Lipid map of the mammalian cell. *J. Cell Sci.* **124**, 5-8.
- Kainu, V. (2012) *Metabolism and Translocation of Aminophospholipids in Mammalian Cells*. Academic dissertation, Faculty of Medicine, University of Helsinki.
- Tallberg, T., Dabek, J., Hallamaa, R. and Atroshi, F. (2011) Lipidomics: the function of vital lipids in embryogenesis preventing autism spectrum disorders, treating sterile inflammatory diatheses with a lymphopoietic central nervous system component. *J. Lipids* **2011**, 137175; doi: 10.1155/2011/137175.
- Kainu, V., Hermansson, M. and Somerharju, P. (2008) Electrospray ionization mass spectrometry and exogenous heavy isotope-labeled lipid species provide detailed information on aminophospholipid acyl chain remodeling. *J. Biol. Chem.* **283**, 3676-3687.
- Hallamaa, R.E. (2009) Characteristics of equine summer eczema with emphasis on differences between Finnhorses and Icelandic horses in a 11-year study. *Acta Vet. Scand.* **51**, 29; doi: 10.1186/1751-0147-51-29.
- Hermansson, M., Uphoff, A., Kälälä, R. and Somerharju, P. (2005) Automated quantitative analysis of complex lipidomes by liquid chromatography/mass spectrometry. *Anal. Chem.* **77**, 2166-2175.
- Subbaiah, P.V. and Liu, M. (1996) Comparative studies on the substrate specificity of lecithin:cholesterol acyltransferase towards the molecular species of phosphatidylcholine in the plasma of 14 vertebrates. *J. Lipid Res.* **37**, 113-122.
- Fuchs, B., Bondzio, A., Wagner, U. and Schiller, J. (2009) Phospholipid compositions of sera and synovial fluids from dog, human and horse: a comparison by ³¹P-NMR and MALDI-TOF MS. *J. Anim. Physiol. Anim. Nutr.* **93**, 410-422.
- O'Connor, C.I., Lawrence, L.M. and Hayes, S.H. (2007) Dietary fish oil supplementation affects serum fatty acid concentrations in horses. *J. Anim. Sci.* **85**, 2183-2189.
- Kierszenbaum, A.L. (2002) Cytomembranes. In: *Histology and Cell Biology*, Ed: A.L. Kierszenbaum, Mosby, Inc., Elsevier Science, St Louis. pp 54-75.
- Oliver, A. and Rivera, J. (2011) An emerging role for the lipid mediator sphingosine-1-phosphate in mast cell effector function and allergic disease. *Adv. Exp. Med. Biol.* **716**, 123-142.
- Proksch, E., Jensen, J.-M. and Elias, P.M. (2003) Skin lipids and epidermal differentiation in atopic dermatitis. *Clin. Dermatol.* **21**, 134-144.