

Phospholipid Esters from Herring Roe promotes SPM biosynthesis in human monocyte-derived macrophages with implications for the treatment of psoriasis



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Background

Phospholipid Esters from Herring Roe (PEHeRo) are polar amphipathic lipids naturally enriched in marine long-chain polyunsaturated fatty acids (LC-PUFAs). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the most abundant omega-3 LC-PUFAs in PEHeRo and have known involvement in the resolution of inflammation through specialized pro-resolving mediator (SPM) biosynthesis. Prior studies on Herring Roe Oil (HRO) containing PEHeRo (IRIS ID: 300000046327) have displayed promising immunomodulatory functions in vivo, where HRO has been shown to improve mild-to-moderate psoriasis in a clinical trial in humans (n=64). Psoriasis is a multifactorial inflammatory disease associated with keratinocyte hyperproliferation and elevated inflammatory cytokine levels, where the IL-23/IL-17 axis is central.

Scope of current work:

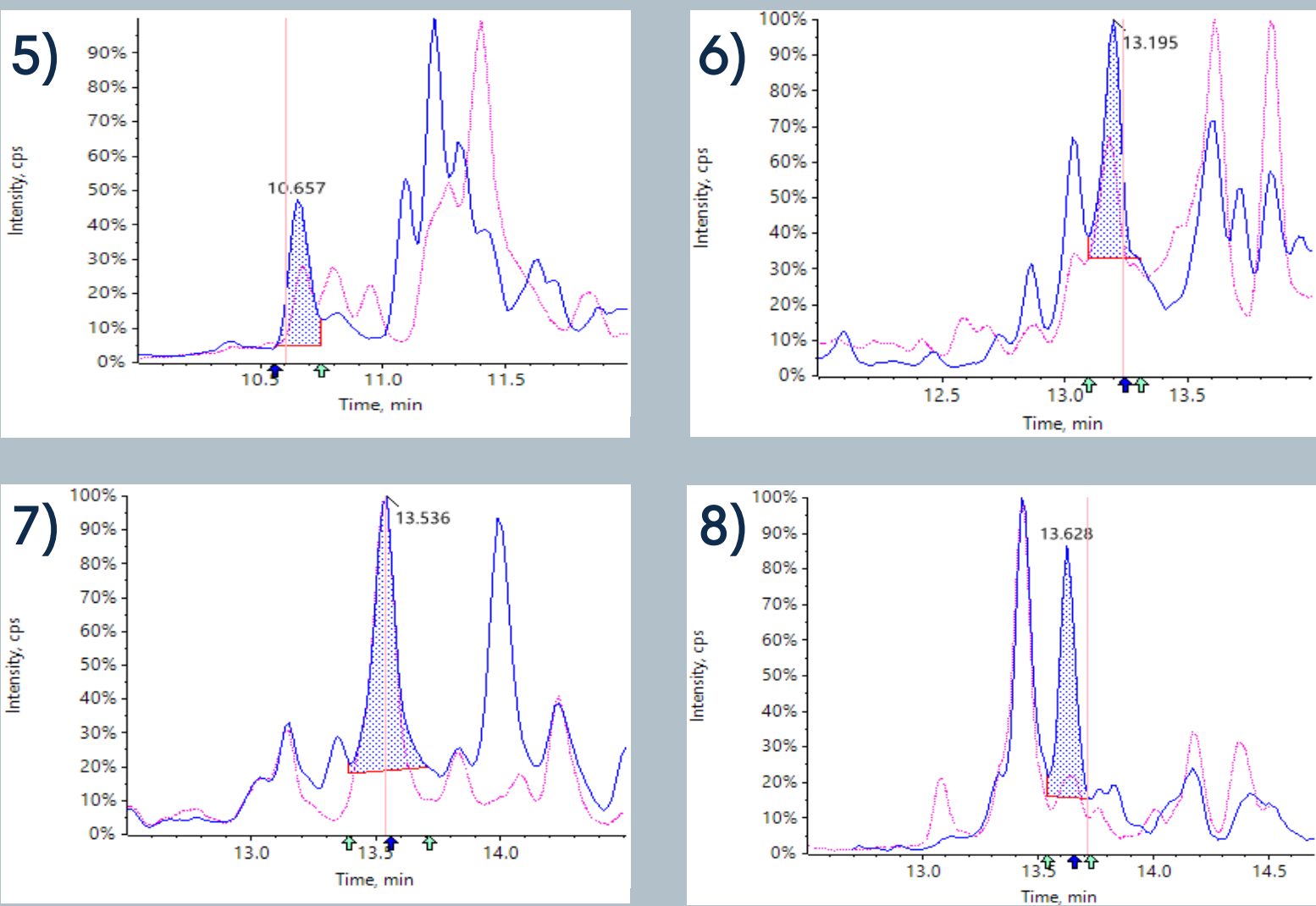
We have investigated the effect of HRO and PEHeRo on pro-resolving biosynthetic pathways in interleukin-23 (IL-23) producing activated human monocyte-derived macrophages (MDM).

Results and conclusion

Key results:

1. Marked upregulation of SPM biosynthesis from EPA, DHA, and n-3 DPA in activated MDMs after stimulation with HRO and PEHeRo
2. Upregulation of SPMs reported to be relevant for resolution of inflammation on the IL-23/IL-17 axis
3. Overall composition of the oils influences the promotion of SPM biosynthesis

Chromatograms:

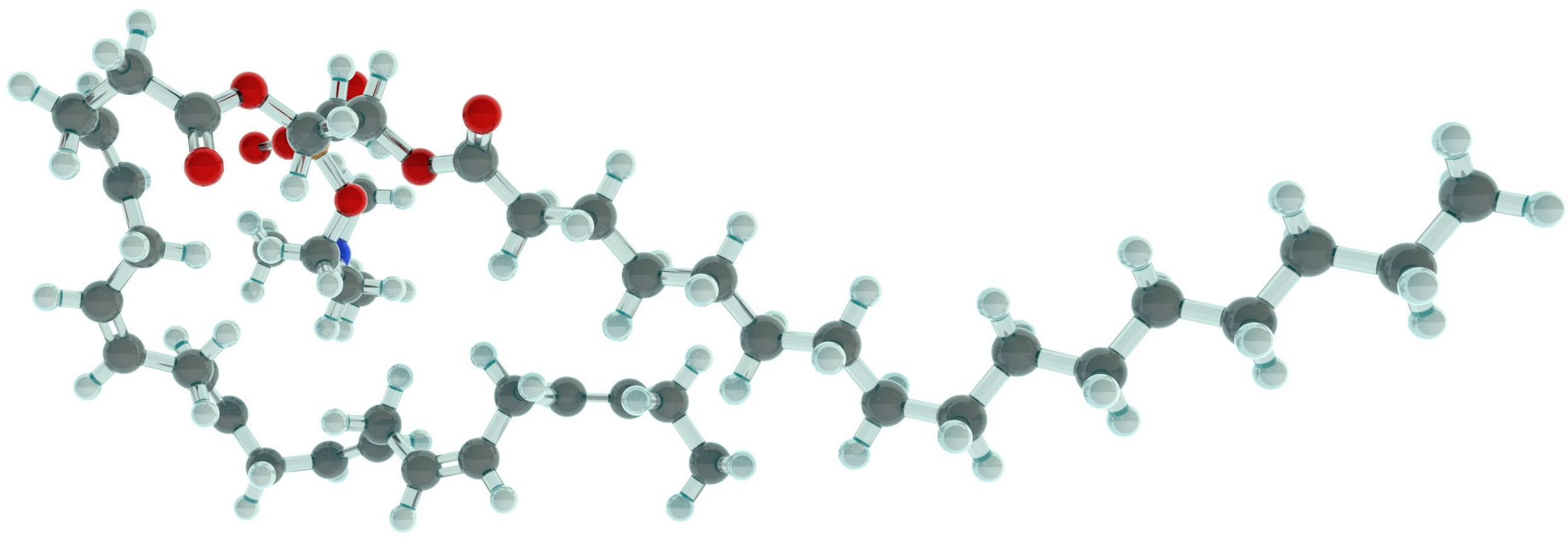


5) RvD2 in PEHeRo 0.25% + LPS 6) PDx in PEHeRo 0.25% + LPS 7) RvE3 in HRO 0.05% + LPS 8) RvD5_{n-3} DPA in HRO 0.05% + LPS

Conclusion:

Our findings support an anti-inflammatory action of HRO, and PEHeRo specifically, through upregulation of SPMs that promote a shift in the MDM phenotype towards a protective and possibly reparative one. This effect could be a promising treatment modality in inflammatory conditions, including psoriasis.

PEHeRo contains a complex mixture of biologically relevant phospholipids



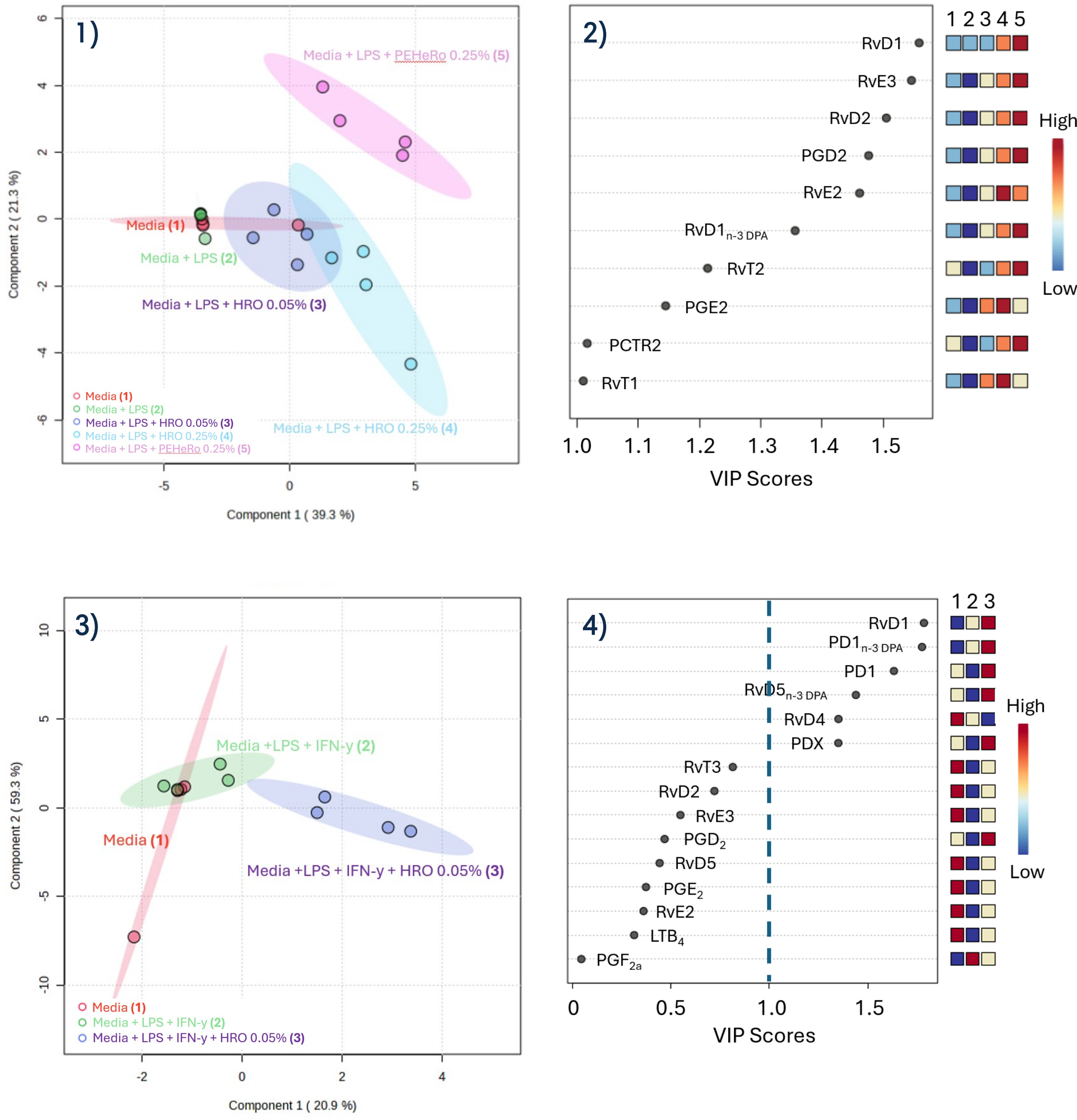
SPMs in the activated MDM cell supernatants after stimulation (co-stimulation or pretreatment) with HRO or PEHeRo were quantified using LC-MS/MS and analyzed using partial least squares discriminant analysis (PLS-DA) as shown in Figs. 1-4.

Co-stimulation with LPS (Fig. 1 and Fig. 2) shows significant and dose-dependent upregulation of EPA (RvE3 and RvE2), DHA (RvD1, RvD2, PDx, and PCTR2), and n-3 DPA (RvD1_{n-3} DPA and RvT2) SPMs that indicates a shift towards a more protective and reparative phenotype of the MDMs. The reparative activity is indicated through activation of the cysteinyl SPM pathway for PCTR2s, that has been reported to be involved in tissue regeneration.¹ Upregulation of SPMs indicative of an MDM phenotype shift is also observed when the MDMs are pretreated with the oils, albeit with a slightly different qualitative profile and generally lower magnitude of SPM expression.

Interestingly, the MDMs in all the experiments displayed upregulation of SPMs from the n-3 DPA metabolome which was unexpected given the levels of n-3 DPA in HRO/PEHeRo. It should also be noted that supernatant SPM profiles are vastly different to the lipid mediator profiles in the oils, thus excluding carry-over of lipid mediators from stimulation as an origin to the observed SPMs.

The PLA-DA analysis in Fig. 1 also shows that there is a difference between HRO and purified PEHeRo, with a higher abundance of several SPMs (RvD1, RvD2, PCTR2, and RvE3) when stimulated with PEHeRo compared to HRO. This difference cannot be explained by normalization based on fatty acids or mono-hydroxylated precursors in the oils and suggests that the overall composition of the oils, and not only levels of individual fatty acids, influences the SPM activity observed in the MDMs.

The observed results are considered relevant for the treatment of psoriasis with PEHeRo as RvD1, RvE2, and PDx has been implicated in the resolution of inflammation in psoriasis and RvE3 has been reported to attenuate inflammation via the IL-23/IL-17A axis.²⁻⁵



1) Partial least squares discriminant analysis (PLS-DA) of MDMs incubated with or without LPS and HRO/PEHeRo 2) Variable importance in projection (VIP) scores for 10 SPMs with greatest contribution to difference between the groups in 1. 3) PLS-DA analysis of MDMs incubated with or without LPS+IFN γ and HRO. 4) VIP scores for 15 SPMs contributing to difference between the groups in 3.

References

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Materials and methods

Preparation of HRO: Immature herring roe (IHR) was dried under reduced pressure (until H₂O <20% w/w), then contacted with EtOH/H₂O to extract the lipids. Centrifugation of the resulting suspension yielded a clarified lipid extract which then was concentrated under reduced pressure, desalinated by crystallization in abs. EtOH, and filtered to give a desalted lipid concentrate. The final HRO was prepared by standardization and viscosity modification through addition of a reconstituted triglyceride oil and evaporation under reduced pressure. **Preparation of PEHeRo:** IHR was contacted with EtOH/H₂O to extract lipids, before the resulting extraction was clarified by centrifugation and dried under reduced pressure. The dried crude extract was then desalinated by crystallization in abs. EtOH and filtration. PEHeRo (>90% w/w dry matter) was purified from the desalinated extract using normal-phase flash column chromatography (EtOH/H₂O 88:12, 200 nm). **Oil preparation for cell treatment:** The oils were freshly prepared as aqueous emulsions (5% w/w) from dH₂O by stirring under inert atmosphere and pasteurized by sonication. **Cell cultures:** Buffy coats from 4 donors were obtained from the blood bank Ålesund (Helse Møre og Romsdal, Norway) with approval by the Regional Ethics Committee (REK, ref.: 230804). PBMC were isolated from buffy coats by a LymphoprepTM density gradient and monocytes were selected by plastic adherence in 6-well plates and differentiated into monocyte-derived macrophages (MDM), followed by incubation without growth factors and cytokines for 2 days. MDMs from each donor were treated in 1.5 ml per well with indicated final percentages (w/w) of the different oils. MDMs were either co-stimulated with 1 ng/ml lipopolysaccharide (LPS) for 24 h or treated with oils for 16 h, followed by change of medium with addition of LPS and IFN- γ for further 24 h. Supernatants were collected from 3 wells per condition and pooled, centrifuged and frozen at -80°C until analysis. **LM analysis:** Proteins were precipitated using two volumes of ice-cold methanol containing deuterated internal standards. The latter were employed to facilitate lipid mediator identification and quantitation. Lipid mediators were extracted using C18 SPE and identified and quantified using LC-MS/MS. Each lipid mediator was identified using the following criteria: (1) matching retention time to synthetic or authentic standards (\pm 0.05 min), (2) signal-to-noise ratio \geq 5 for a primary transition and (3) signal-to-noise ratio of \geq 3 for a secondary transition.