Palmitoleic Acid-Containing Phospholipids as Modulators of Immunometabolic Pathways

Jesús Balsinde

Consejo Superior de Investigaciones Científicas (CSIC), and CIBERDEM. Valladolid, Spain

July 2, 2026

Palmitoleic acid (16:1n-7) has long been proposed to exert beneficial metabolic and anti-inflammatory effects, yet its molecular mode of action remains unclear. We investigated how phagocytic cells handle palmitoleic acid and found that ~50% of the cellular pool is concentrated in a single phospholipid species, PC(16:0/16:1). Delivering this intact phospholipid to macrophages strongly suppressed LPS-induced inflammatory responses, matching or exceeding the effects of the free fatty acid. Using pharmacological inhibition, antisense depletion of iPLA$_2$β, and a phospholipase-resistant ether analog, we demonstrated that biological activity does not require release of free palmitoleic acid, indicating that the intact phospholipid is the bioactive form. PC(16:0/16:1) reduced NF-κB p65 nuclear translocation, up-regulated M2-associated markers (Ym1, Fizz1, Mrc1), and enhanced phagocytic capacity. Together, these findings show that PC(16:0/16:1) drives macrophages toward an anti-inflammatory, pro-resolving phenotype, highlighting the therapeutic potential of defined lipid species in modulating innate immune function.

Transcription of the lecture presented on Thursday July 2, 2026 at the FATS-O-MICS Workshop on Food Lipids and Lipidomics for Health, Nutrition, and Sustainability, held in Aveiro, Portugal (**Slide 1**).

Good afternoon everyone, and thanks for inviting me to speak here today. I have to say that I am not actually in the nutrition field so I hope I will learn lots of useful things throughout this workshop. Today I am going to talk about palmitoleic acid, a monounsaturated fatty acids that has been in the spotlight of lipid signaling for quite a long time now, owing to its properties as a hormone-like that, traveling from the adipose tissue to the liver, can reduce liver steatosis and improve insulin sensitivity (**Slide 2** – Lipid Inflammatory Signals Regulate Cellular Lipid Metabolism) Because of this and a general anti-inflammatory effect, palmitoleic acid has found wide use as a nutraceutical. I do not know if you can see the label: The New Good Fat… well, palmitoleic acid has been used as the new good fat for quite some time…

Of course, as it happens with so many other findings, many of the good things attributed to palmitoleic acid have been confirmed, and others have not, and conflicting evidence of its benefits still exists, particularly in humans. Much of this is due to the fact that molecular details of the mechanisms through which palmitoleic acid acts remain unknown.

Quite a few years ago we became interesed in palmitoleic acid and decided to investigate its

molecular effects in the innate immune system (**Slide 3** – What Is the Biological Relevance of Palmitoleic Acid in Phagocytic Cells?). We demonstrated that loading innate immune cells with palmitoleic acid reduced the inflammatory potential of the cells as can be seen here (**Slide 4** – Palmitoleic Acid Possesses Anti-inflammatory Properties in vitro), where cells loaded with palmitoleic acid show reduced responses to bacterial lipopolysaccharide, and the effects are comparable to those of DHA, and omega-3 fatty acid. And this anti-inflammatory effect could be also be observed in mice, in a simple model of i.p. injection of LPS, animals that had received palmitoleic acid also showed reduced production of the pro-inflammatory cytokine IL-6, both at the RNA and protein levels. In animals, the palmitoleic acid effects were even stronger than those of DHA (**Slide 5** – Palmitoleic Acid Possesses Anti-inflammatory Properties in vivo).

Well, we are lipid biochemists, so the key question we wanted to address is how cells handle their palmitoleic acid content, in particular where they place it, and how they use it. (**Slide 6** – Cellular Utilization of 16:1 Fatty Acids by Phagocytic Cells). So we conducted a full lipidomic analysis of the cells by mass spectrometry, both GC-MS and LC-MS (**Slide 7** – Distribution of Palmitoleic Acid Among Phospholipids). The first thing that caught our attention is that the vast majority of 16:1 fatty acids are localized in PC phospholipids; minor amounts in PE, PI or PS, and lots in PC. Not only that, when we analyzed species by LC-MS, we found that ~50% of total cellular palmitoleic acid is contained within one single species, PC(16:0/16:1). This is unusual and seems to suggest that such a compartmentalization may have some biological meaning.

So it got us thinking: what would happen if, instead of adding the free fatty acid, we added that phospholipid instead, the whole phospholipid? This makes sense, because if we think about the posible therapeutic potential of palmitoleic acid, delivery of a phospholipid is preferable over that of a fatty acid because of toxicity issues. We synthesized in the lab the required lipid, and added it to the macrophages (**Slide 8** – Chemical structures of Phospholipids). We also synthesized two phospholipids without palmitoleic acid for use as controls. We worked out the conditions for the incorporation of the phospholipids to the macrophages, and we got conditions that results in the cells taking them up quite well; the cells take up the phospholipid and retain it in its original form, and metabolism is negligible.

We exposed the cells to LPS and measured IL-6 and TNF-α expression at both gene and protein expression levels; you can there is a strong inhibitory effect. Note that we also used phospholipids containing oleic acid or palmitic acid as controls, and neither of these exerted any significant effect (**Slide 9** – Anti-inflammatory activity of AA-containing PC).

So, the conclusion here is that adding the whole phospholipid works pretty much as well as adding the free fatty acid. Then, the next queston here is: what is the bioactive form of palmitoleic acid, the whole phospholipid, or the free fatty acid that could be released from that species? (**Slide 10** – What Is the Bioactive Form of Palmitoleic Acid?). Like many phospholipids containing a palmitic acid tail at sn-1, PC(16:0/16:1) is an excellent substrate for a particular intracellular phospholipase A_2, one that is called iPLA$_2$β, which catalyzes the generation of free palmitoleic acid from cells. So we conducted studies in the presence of

inhibitors of iPLA$_2$β, which completely prevent the release of the fatty acid from the phospholipid. And, as you can see here, the biological effect, the anti-inflammatory effect, is maintained. These data suggested that PC(16:0/16:1) hydrolysis by iPLA$_2$β is not required for an anti-inflammatory effect.

To substantiate this suggestion, we prepared cells deficient in iPLA$_2$β by using antisense inhibition, which is a well established approach. You see again that, when challenged with LPS, both iPLA$_2$β-deficient and normal cells exhibited similar inhibitory responses to PC(16:0/16:1n−7), underscoring the lack of iPLA$_2$β involvement in this effect (**Slide 10** – What Is the Bioactive Form of Palmitoleic Acid?).

To obtain further evidence, we went on to synthesize a PC molecule in which the palmitoleic acid lateral chain is linked to the glycerol backbone not by a classical ester bond but by an ether bond, PC(16:0/O-16:1) (**Slide 10** – What Is the Bioactive Form of Palmitoleic Acid?). This modification makes the phospholipid resistant to phospholipase attack and, hence, unable to release the palmitoleic acid moiety. And… you can see here that, when used under identical conditions, both PC(16:0/16:1) and the ether analog, PC(16:0/O-16:1), inhibited the LPS-induced gene expression to comparable levels. Thus, it seems it is the whole phospholipid, not the free fatty acid that conveys biological activity.

Nuclear factor kappa B (NF-κB) plays a central role in regulating the transcription of proinflammatory genes in macrophages. When activated, the p65/p50 heterodimer of NF-κB translocates to the nucleus, where it drives gene expression. So we measured p65 translocation in macrophages by immunofuorescence. Blue is DAPI which marks the nucleus, p65 staining is in red, and co-localization is white. I know the figure is hard to see, but we have the quantification on the right hand side that can help a bit (**Slide 11** – 16:1-containing PC Inhibits NFκB translocation). Immunofluorescence staining of p65 in unstimulated macrophages showed largely a cytoplasmic location; you see there are no white spots at all. However when treated with LPS you see the white spots, indicating that p65 translocated to the nucleus, and these white spots are greatly reduced in the PC(16:0/16:1)-enriched cells.

Given that NF-κB is a hallmark of proinflammatory gene expression, we speculated that reduced activity of this transcription factor would conceivably skew the cells to a more pronounced anti-inflammatory character. Thus we decided to analyze the expression of two widely used markers for alternatively activated macrophages Ym1 (Chi3l3) and Fizz1 (Retnla) by qPCR (**Slide 12** – Polarized Activation of Macrophages). Both markers were found to be markedly up-regulated in the PC(16:0/16:1)-loaded macrophages compared to untreated cells (**Slide 13** – 16:1-containing PC Promotes an Anti-inflammatory Profile). Together, these results suggest that loading the macrophages with 16:1-containing PC intensifies an anti-inflammatory character.

To further characterize the PC(16:0/16:1) effects, we conducted transcriptional mRNA profiling in murine macrophages under conditions that lead to an anti-inflammatory M2-like phenotype (IL-13 plus IL-4, 20 ng/ml, each for 12 h). To assess gene expression changes

associated with M2 polarization, we analyzed a number of genes widely characterized to increase during M2 polarization by RNAseq in cells incubated with or without phospholipid (**Slide 13** – 16:1-containing PC Promotes an Anti-inflammatory Profile). The findings clearly demonstrated that PC(16:0/16:1) substantially enhance the anti-inflammatory properties of macrophages by modulating gene expression patterns associated with alternative polarization. One of the genes in this list, *Mrc1*, codes for CD206, a mannose receptor. Flow cytometry analyses of the expression of this receptor confirmed it is increased in the 16:1-containing PC-treated cells (**Slide 13** – 16:1-containing PC Promotes an Anti-inflammatory Profile).

Enhanced phagocytosis is a hallmark of alternatively activated (M2) macrophages, setting them apart from their pro-inflammatory counterparts. Therefore, we aimed to assess next whether loading macrophages with 16:1-containing PC would induce a heightened phagocytic response. The 16:1-containing PC-loaded macrophages were exposed to fluorescent yeast-derived zymosan as a phagocytic challenge, and analyzed by confocal microscopy (**Slide 14** – 16:1-containing PC Enhances Zymosan Phagocytosis). Cells loaded with PC(16:0/16:1) manifested a significantly enhanced phagocytic response. Parallel experiments utilizing cells loaded with either PC(16:0/18:1) or PC(16:0/16:0) showed no enhanced phagocytic activity compared to otherwise untreated control cells, thus highlighting the specificity of action of the palmitoleic moiety.

So, as a summary, the only contribution of an AI to my presentation, a slide that summarizes everything that I have been telling you: palmitoleic acid being bioactive as long as it is part of PC(16:0/16:1), promoting macrophage proliferation towards an anti-inflammatory, potentially pro-resolutory state which underscores the pharmacotherapeutic potential of defined lipid species in reprogramming macrophage function in inflammatory diseases (**Slide 15** – A Bioactive Key to Unlocking Macrophage Anti-inflammatory Functions).

To conclude, thanks to the palmitoleic crew of my lab… (**Slide 16** – Acknowledgments). Thanks as well to our collaborators and our sponsors (**Slide 17** – Acknowledgments)... As requested, a comprehensive list of significant papers from our laboratory follows.

REFERENCES

1. Guijas, C., G. Pérez-Chacón, A. M. Astudillo, J. M. Rubio, L. Gil-de-Gómez, M. A. Balboa, and J. Balsinde. 2012. Simultaneous activation of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A_2-dependent synthesis of lipid droplets in human monocytes. *J. Lipid Res*. 53: 2343–2354.
2. Guijas, C., C. Meana, A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2016. Foamy monocytes are enriched in cis-7-hexadecenoic fatty acid (16:1n-9), a possible biomarker for early detection of cardiovascular disease. *Cell Chem. Biol*. 23: 689–699.
3. Astudillo, A. M., C. Meana, C. Guijas, L. Pereira, R. Lebrero, M. A. Balboa, and J. Balsinde. 2018. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *J. Lipid Res*. 59: 237–249.
4. Astudillo, A.M., C. Meana, M.A. Bermúdez, A. Pérez-Encabo, M.A. Balboa, and J. Balsinde. 2020. Release of anti-inflammatory palmitoleic acid and its positional isomers by mouse peritoneal macrophages.

Biomedicines 8: 480.
5. Bermúdez, M.A., A. Garrido, L. Pereira, T. Garrido, M.A. Balboa, and J. Balsinde. 2024. Rapid movement of palmitoleic acid from phosphatidylcholine to phosphatidylinositol in activated human monocytes. *Biomolecules* 14: 707.
6. Bermúdez, M.A., C. Meana, A. Garrido, A. Pérez-Encabo, Balboa, and J. Balsinde. 2025. Phosphatidylcholine-bound palmitoleic acid: a bioactive key to unlocking macrophage anti-inflammatory functions. *Biomed. Pharmacother*. 192: 118652.
7. Balsinde, J., M. V. Winstead, and E. A. Dennis. 2002. Phospholipase A_2 regulation of arachidonic acid mobilization. *FEBS Lett*. 531: 2-6.
8. Pérez-Chacón, G., A. M. Astudillo, D. Balgoma, M. A. Balboa, and J. Balsinde. 2009. Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103-1113.
9. Astudillo, A. M., D. Balgoma, M. A. Balboa, and J. Balsinde. 2012. Dynamics of arachidonic acid mobilization by inflammatory cells. *Biochim Biophys. Acta* 1821: 249-256.
10. Guijas, C., J.P. Rodríguez, J.M. Rubio, M.A. Balboa, and J. Balsinde. 2014. Phospholipase A_2 regulation of lipid droplet formation. *Biochim. Biophys. Acta* 1841: 1661–1671.
11. Astudillo, A.M., M.A. Balboa, and J. Balsinde. 2019. Selectivity of phospholipid hydrolysis by phospholipase A_2 enzymes in activated cells leading to polyunsaturated fatty acid mobilization. *Biochim. Biophys. Acta* 1864: 772–783.
12. Bermúdez M.A., M.A. Balboa, and J. Balsinde. 2021. Lipid droplets, phospholipase A_2, arachidonic acid, and atherosclerosis. *Biomedicines* 9: 1891.
13. Bermúdez, M.A., L. Pereira, C. Fraile, L. Valerio, M.A. Balboa, and J. Balsinde. 2022. Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, in inflammation, metabolic diseases and cancer. *Cells* 11: 2146.
14. Astudillo, A.M., M.A. Balboa, and J. Balsinde. 2023. Compartmentalized regulation of lipid signaling in oxidative stress and inflammation: Plasmalogens, oxidized lipids and ferroptosis as new paradigms of bioactive lipid research. *Prog. Lipid Res.* 89: 101207.
15. Rodríguez, J.P., J. Casas, M.A. Balboa, and J. Balsinde. 2025. Bioactive lipid signaling and lipidomics in macrophage polarization: impact on inflammation and immune regulation. *Front. Immunol.* 16: 1550500.
16. Balsinde, J., and M. A. Balboa. 2005. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A_2 in activated cells. *Cell. Signal.* 17: 1052–1062.
17. Balboa, M. A., J. Balsinde, C. A. Johnson, and E. A. Dennis. 1999. Regulation of arachidonic acid mobilization in lipopolysaccharide-activated $P388D_1$ macrophages by adenosine triphosphate. *J. Biol. Chem*. 274: 36764 –36768.
18. Balboa, M.A., and J. Balsinde. 2021. Phospholipases: from structure to biological function. *Biomolecules* 11: 428.
19. Gil-de-Gómez, L., P. Monge, J.P. Rodríguez, A.M. Astudillo, M.A. Balboa, and J. Balsinde. 2020. Phospholipid arachidonic acid remodeling during phagocytosis in mouse peritoneal macrophages. *Biomedicines* 8: 274.
20. Bermúdez, M.A., J.M. Rubio, M.A. Balboa, and J. Balsinde. 2022. Differential mobilization of the phospholipid and triacylglycerol pools of arachidonic acid in murine macrophages. *Biomolecules* 12: 1851.
21. Monge, P., Garrido, A., Rubio, J.M., Magrioti, V., Kokotos, G., Balboa, M.A. & Balsinde, J. 2020. The contribution of cytosolic group IVA and calcium-independent group VIA phospholipase A_2s to adrenic acid mobilization in murine macrophages. *Biomolecules* 10: 542.
22. Monge, P., A.M. Astudillo, L. Pereira, M.A. Balboa, J. Balsinde. 2023. Dynamics of docosahexaenoic acid utilization by mouse peritoneal macrophages. *Biomolecules* 13: 1635.
23. Gil-de-Gómez, L., A. M. Astudillo, P. Lebrero, M. A. Balboa, and J. Balsinde. 2017. Essential role for ethanolamine plasmalogen hydrolysis in bacterial lipopolysaccharide priming of macrophages for enhanced arachidonic acid release. *Front. Immunol*. 8: 1251.
24. Guijas, C., M.A. Bermúdez, C. Meana, A.M. Astudillo, L. Pereira, L. Fernández-Caballero, M.A. Balboa, and J. Balsinde. 2019. Neutral lipids are not a source of arachidonic acid for lipid mediator signaling

in human foamy monocytes. *Cells* 8: 941.
25. Lebrero, P., A.M. Astudillo, J.M. Rubio, L. Fernández-Caballero, G. Kokotos, M.A. Balboa, and J. Balsinde. 2019. Cellular plasmalogen content does not influence arachidonic acid levels or distribution in macrophages: a role for cytosolic phospholipase $A_2\gamma$ in phospholipid remodeling. *Cells* 8: 799.
26. Peña, L., C. Meana, A. M. Astudillo, G. Lordén, M. Valdearcos, H. Sato, M. Murakami, J. Balsinde, and M. A. Balboa. 2016. Critical role for cytosolic group IVA phospholipase A_2 in early adipocyte differentiation and obesity. *Biochim. Biophys. Acta* 1861: 1083–1095.
27. Garrido, A., P. Monge, N. Pérez, M.A. Balboa, and J. Balsinde. 2026. Incorporation of hydroxyeicosatetraenoic acid isomers into macrophage phospholipids reveals class-specific distribution. Biomolecules 16: 692.
28. Barceló-Coblijn, G., J. Balsinde. 2025. Advances in lipid research: from bench to bedside. A special issue dedicated to the 63rd International Conference on the Bioscience of Lipids. Biochim. Biophys. Acta 1870: 59624.

THE EICOSANOID
RESEARCH DIVISION
VALLADOLID