Transferencia de ácido palmitoleico desde fosfatidilcolina a fosfatidilinositol en monocitos humanos

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En este trabajo se describe una nueva ruta para la remodelación de ácidos grasos en fosfolípidos que involucra al ácido palmitoleico, un ácido graso monoinsaturado. Cuando se añade a monocitos humanos, el ácido palmitoleico se incorpora rápidamente en fosfolípidos de membrana, en particular en fosfatidilcolina (PC). En células en reposo, el ácido palmitoleico permanece en de los fosfolípidos donde se incorporó inicialmente y no demuestra ulteriores movimientos. Sin embargo, la estimulación de los monocitos humanos con agonistas dirigidos a receptor (zimosán opsonizado) o solubles (ionóforo de calcio A23187) da como resultado la rápida transferencia de ácido palmitoleico de PC a fosfatidilinositol (PI). Esto se debe a la activación de una ruta de remodelación dependiente de coenzima A que involucra dos fosfolipasas A_2 diferentes que actúan sobre diferentes sustratos para generar ácido palmitoleico libre y aceptores de lisoPI. El enriquecimiento estimulado de especies moleculares de PI con ácido palmitoleico revela una vía hasta ahora no descrita para el recambio de lípidos en monocitos humanos que podría desempeñar un papel importante en la regulación de la señalización por lípidos durante la activación inmune innata.

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El presente estudio ha examinado la incorporación y remodelación de ácido palitoleico (POA) entre las diferentes clases de fosfolípidos durante la activación de monocitos de sangre periférica humana. En los monocitos en reposo, POA se incorpora principalmente en PC, seguido de PE, PI y PS. Esta distribución no cambia con el tiempo, es decir, el POA permanece en la clase de fosfolípidos que lo incorporó inicialmente. Sin embargo, es importante destacar que la remodelación de los ácidos grasos entre fosfolípidos que implican a este ácido graso ocurre después de que las células son estimuladas por agonistas dirigidos a receptor (zimosán) o solubles (ionóforo de calcio). Por tanto, hay un movimiento estimulado de restos de POA de PC a PI. Si bien esta transferencia no cambia drásticamente la distribución de POA entre los fosfolípidos celulares, es decir, la PC sigue siendo el principal fosfolípido que contiene POA en las células, hay un enriquecimiento con POA de dos especies moleculares de PI, a saber, PI(18:0/16:1) y PI(18:1/16:1). Dichos enriquecimientos aumentan la masa celular de PI(18:0/16:1) y PI(18:1/16:1) en aproximadamente 3,1 y 3,8 veces respectivamente. Por tanto, parece probable que estas dos especies puedan desempeñar funciones reguladoras importantes durante la ejecución de respuestas por parte de los monocitos activados. Claramente, el trabajo futuro debería dedicarse a identificar las respuestas celulares que implican a estas especies de PI, de modo que puedan incluirse en el creciente número de especies moleculares de fosfolípidos con funciones específicas en los procesos de activación celular. Si bien abordar estas respuestas celulares está más allá del alcance del trabajo actual, vale la pena mencionar aquí que se ha descrito que al menos una de estas especies, a saber, PI(18:0/16:1), posee

propiedades similares a las de un factor de crecimiento cuando se agrega a fibroblastos [90]. Más recientemente, se ha descrito que la especie relacionada PI(18:1/18:1), aunque carece de POA, vincula el metabolismo de los ácidos grasos insaturados con la señalización de estrés.

El análisis de la ruta metabólica que regula la formación de especies moleculares que contienen POA en monocitos activados revela que procede vía reciclaje de ácidos grasos a través de fosfolipasa A_2/aciltransferasas dependientes de CoA, es decir, el ciclo de Lands, en lugar de hacerlo a través de transferencia directa e independiente de coenzima A, como ocurre predominantemente cuando el ácido graso que se va a remodelar es un poliinsaturado como el ácido araquidónico. Es importante destacar que la vía parece utilizar dos enzimas fosfolipasa A_2 diferentes; la primera proporciona el ácido graso libre y la segunda proporciona el aceptor de lisoPI. Por tanto, esta ruta constituye un excelente ejemplo de la interacción de diferentes enzimas fosfolipasa A_2, que actúan por separado pero en conjunto, dentro de las vías de señalización de lípidos. Utilizando diferentes sustratos, estas fosfolipasas A_2 cooperan para generar variedad en los lípidos, lo que posiblemente ayude a la célula a responder adecuadamente a los estímulos.

Aunque el movimiento de POA entre PC y PI parece ser un proceso cuantitativamente importante, también es posible que se produzcan otras reacciones al mismo tiempo. Nuestros estudios anteriores demostraron que el POA tiende a acumularse en lípidos neutros cuando los monocitos pasan a células espumosas y, en los macrófagos peritoneales de ratón, el POA se utiliza para la formación del éster antiinflamatorio ramificado hidroxiestearato de palmitoleilo. No se han detectado estas reacciones en los monocitos humanos, lo que probablemente se deba, por un lado, al hecho de que los monocitos humanos contienen cantidades considerablemente menores de POA que los macrófagos peritoneales de ratón, por otro lado, a la diferencias de tipo que surgen de roles especializados en fisiología y fisiopatología.

Es de especial interés la participación de $iPLA_2\beta$ como enzima iniciadora de la vía que conduce a la formación elevada de especies de PI enriquecidas con POA. A diferencia de otros miembros de la superfamilia de fosfolipasas A_2, tales como las $sPLA_2$ o $cPLA_2\alpha$, $iPLA_2\beta$ parece carecer de un "interruptor activador" que aumente su actividad celular después de la estimulación, ya sea aumentando la masa de enzima o la actividad enzimática intrínseca. Si bien la $iPLA_2\beta$ no manifiesta ninguna preferencia evidente de sustrato cuando su actividad se determina en ensayos in vitro, estudios con cultivos celulares primarios, células que sobreexpresan $iPLA_2\beta$ o células de animales knock-out para $iPLA_2\beta$, han establecido que $iPLA_2\beta$ manifiesta en las células una clara preferencia por hidrolizar especies de PC que contienen ácido palmítico en la posición sn-1. Esto concuerda perfectamente con los datos de este estudio y se alinea con nuestra hipótesis de que las especificidades de sustrato de las fosfolipasas A_2 observadas in vivo, pueden verse limitadas por su localización subcelular. Por tanto, la compartimentación de sustrato puede constituir un mecanismo importante para la regulación celular de la actividad de $iPLA_2\beta$ sobre los fosfolípidos de membrana.

REFERENCES

1. Franz, M.J. Weight management: obesity to diabetes. Diabetes Spctr. 2017, 30, 149–153.
2. Piché, M.E.; Tchernof, A.; Després, J.P. Obesity phenotypes, diabetes, and cardiovascular diseases. Circ. Res. 2020, 126, 1477–1500.
3. Wyatt, S.B.; Winters, K.P.; Dubbert, P.M. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Am. J. Med. Sci. 2006, 331, 166–174.
4. Hotamisligil, G.S. Inflammation, metaflammation and immunometabolic disorders. Nature 2017, 542; 177–185.
5. Moore, K. J., Tabas, I. Macrophages in the pathogenesis of atherosclerosis. Cell 2011, 145, 341-355.
6. Tabas, I.; Bornfeldt, K.E. Macrophage phenotype and function in different stages of atherosclerosis. Circ. Res. 2016, 118, 653–667.

7. Li, H.; Meng, Y.; He, S.; Tan, X.; Zhang, Y.; Zhang, X.; Wang, L.; Zheng. W. Macrophages, chronic inflammation, and insulin resistance. Cells 2022, 11, 3001.
8. Russo, S.; Kwiatkowski, M.; Govorukhina, N.; Bischoff, R.; Melgert, B.N. Meta-inflammation and metabolic reprogramming of macrophages in diabetes and obesity: the importance of metabolites. Front. Immunol. 2021, 12, 746151.
9. Lauterbach, M.A.; Wunderlich, F.T. Macrophage function in obesity-induced inflammation and insulin resistance. Pflugers Arch. 2017, 469, 385-396.
10. Mukherjee, S.; Skrede, S.; Haugstøyl, M.; López, M.; Fernø J. Peripheral and central macrophages in obesity. Front. Endocrinol. 2023, 14, 1232171.
11. Chavakis, T.; Alexaki, V.I.; Ferrante, A.W., Jr. Macrophage function in adipose tissue homeostasis and metabolic inflamma-tion. Nat. Immunol. 2023, 24, 757–766.
12. Santa-María, C.; López-Enríquez, S.; Montserrat-de la Paz, S.; Geniz, I.; Reyes-Quiroz, M.E.; Moreno, M.; Palomares, F.; So-brino, F.; Alba, G. Update on anti-inflammatory molecular mechanisms induced by oleic acid. Nutrients 2023, 15, 224.
13. López, S.; Bermúdez, B.; Montserrat-de la Paz, S.; Jaramillo, S.; Varela, L.M.; Ortega-Gómez, A.; Abia, R.; Muriana, F.J.G. Membrane composition and dynamics: a target of bioactive virgin olive oil constituents. Biochim. Biophys. Acta 2014, 1838, 1638–1656.
14. Dernini, S.; Berry, E.M. Mediterranean diet: from a healthy diet to a sustainable dietary pattern. Front. Nutr. 2015, 2, 1–7.
15. Schwingshackl, L.; Hoffmann, G. Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. Nutrients 2012, 4, 1989–2007.
16. Cao, H.; Gerhold, K.; Mayers, J.R.; Wiest, M.M.; Watkins, S.M.; Hotamisligil, G.S. Identification of a lipokine, a lipid hormone 440 linking adipose tissue to systemic metabolism. Cell 2008, 134, 933–944.
17. Erbay, E.; Babaev, V.R.; Mayers, J.R.; Makowski, L.; Charles, K.N.; Snitow, M.E.; Fazio, S.; Wiest, M.M.; Watkins, S.M.; Lin-ton, M.F.; et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat. Med. 2009, 15, 1383–1391.
18. Cetin, E.; Pedersen, B.; Porter, L.M.; Adler, G.K.; Burak, M.F. Protocol for a randomized placebo-controlled clinical trial using pure palmitoleic acid to ameliorate insulin resistance and lipogenesis in overweight and obese subjects with prediabetes. Front. Endocrinol. 2024, 14, 1306528.
19. de Souza, C.O.; Vannice, G.K.; Rosa Neto, J.C.; Calder, P.C. Is palmitoleic acid a plausible nonpharmacological strategy to 482 prevent or control chronic metabolic and inflammatory disorders? Mol. Nutr. Food Res. 2018, 62, 1700504.
20. Bermúdez, M.A.; Pereira, L.; Fraile, C.; Valerio, L.; Balboa, M.A.; Balsinde, J. Roles of palmitoleic acid and its positional iso-mers, hypogeic and sapienic ac-ids, in inflammation, metabolic diseases and cancer. Cells 2022, 11, 2146.
21. Chan, K.L.; Pillon, N.J.; Sivaloganathan, D.M.; Costford, S.R.; Liu, Z.; Théret, M.; Chazaud, B.; Klip, A. Palmitoleate reverses high fat-induced proinflammatory macrophage polarization via AMP-activated protein kinase. J. Biol. Chem. 2015, 290, 16979–16988.
22. de Souza, C.O.; Valenzuela, C.A.; Baker, E.J.; Miles, E.A.; Rosa Neto, J.C. Calder, P. C. Palmitoleic acid has stronger an-ti-in-flammatory potential in human endothelial cells compared to oleic and palmitic acids. Mol. Nutr. Food Res. 2018, 62, e1800322.
23. Souza, C.O.; Teixeira, A.A.S.; Biondo, L.A.; Silveira, L.S.; de Souza Breda, C.N.; Braga, T.T.; Camara, N.O.S.; Belchior, T.; Festuccia, W.T.; Diniz, T.A.,; et al. Palmitoleic acid reduces high fat diet-induced liver inflammation by promoting PPAR-γ-independent M2a polarization of myeloid cells. Biochim. Biophys. Acta 2020, 1865, 158776.
24. Tsai, Y.W.; Lu, C.H.; Chang, R.C.; Hsu, Y.P.; Ho, L.T,; Shih, K.C. Palmitoleic acid ameliorates palmitic acid-induced proin-flammation in J774A.1 macrophages via TLR4-dependent and TNF-alpha-independent signallings. Prostag. Leukot. Essent. Fatty Acids 2021, 169, 102270.

25. Guijas, C.; Meana, C.; Astudillo, A.M.; Balboa, M.A.; Balsinde, J. 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. 2016, 23, 689–699.
26. Astudillo, A.M.; Meana, C.; Guijas, C.; Pereira, L.; Lebrero, R.; Balboa, M.A.; Balsinde, J. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. J. Lipid Res. 2018, 59, 237–249.
27. Ntambi, J.M.; Miyazaki, M. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 2004, 43, 91–104. 431.
28. Kamal, S.; Saleem, A.; Rehman, S.; Bibi, I.; Iqbal, H.M.N. Protein engineering: regulatory perspectives of stearoyl CoA de-saturase. Int. J. Biol. Macromol. 2018, 114, 692–699.
29. Astudillo, A.M.; Meana, C.; Bermúdez, M.A.; Pérez-Encabo, A.; Balboa, M.A.; Balsinde, J. Release of anti-inflammatory pal-mitoleic acid and its positional isomers by mouse peritoneal macrophages. Biomedicines 2020, 8, 480.
30. Guijas, C.; Pérez-Chacón, G.; Astudillo, A.M.; Rubio, J.M.; Gil-de-Gómez, L.; Balboa, M.A.; Balsinde, J. Simultaneous activa-tion of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A_2-dependent synthesis of lipid droplets in human monocytes. J. Lipid Res. 2012, 53, 2343–2354.
31. Yore, M.M.; Syed, I.; Moraes-Vieira, P.M.; Zhang, T.; Herman, M.A.; Homan, E.A.; Patel, R.T.; Lee, J.; Chen, S.; Peroni, O.D.; et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 2014, 159, 318–332.
32. Brejchova, K.; Balas, L.; Paluchova, V.; Brezinova, M.; Durand, T.; Kuda, O. Understanding FAHFAs: from structure to metabolic regulation. Prog. Lipid Res. 2020, 79, 101053.
33. Pérez-Chacón, G.; Astudillo, A.M.; Ruipérez, V.; Balboa, M.A.; Balsinde, J. Signaling role for lysophospholipid acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. J. Immunol. 2010, 184, 1071–1078.
34. Rubio, J.M.; Rodríguez, J.P.; Gil-de-Gómez, L.; Guijas, C.; Balboa, M.A.; Balsinde, J. Group V secreted phospholipase A_2 is up-regulated by interleukin-4 in human macrophages and mediates phagocytosis via hydrolysis of ethanolamine phospho-lipids. J. Immunol. 2015, 194, 3327–3339.
35. Balsinde, J.; Balboa, M.A.; Dennis, E.A. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated P388D1 macrophage-like cells. J. Biol. Chem. 2000, 275, 22544–22549.
36. Balboa, M.A.; Pérez, R.; Balsinde, J. Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A_2 is regulated by cytosolic phospholipase A_2-derived hydroperoxyeicosatetraenoic acid. J. Immunol. 2003, 171, 989–994.
37. Balboa, M.A.; Sáez, Y.; Balsinde, J. Calcium-independent phospholipase A_2 is required for lysozyme secretion in U937 promonocytes. J. Immunol. 2003, 170, 5276–5280.
38. Balsinde, J.; Balboa, M.A.; Insel, P.A.; Dennis, E.A. Differential regulation of phospholipase D and phospholipase A2 by pro-tein kinase C in P388D1 macrophages. Biochem. J. 1997, 321, 805–809.
39. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976, 72, 248–254.
40. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 1959, 37, 911–917.
41. Fine, J.B.; Sprecher, H. Unidimensional thin-layer chromatography of phospholipids on boric acid-impregnated plates. J. Lipid Res. 1982, 23, 660–663.
42. Xu, G.; Waki, H.; Kon, K.; Ando, S. Thin-layer chromatography of phospholipids and their lyso forms: application to deter-mination of extracts from rat hippocampal CA1 region. Microchem. J. 1996, 53, 29–33.
43. Fuchs, B.; Süss, R.; Teuber, K.; Eibisch, M.; Schiller, J. Lipid analysis by thin-layer chromatography--a review of the current state. J. Chromatogr. A 2011, 1218, 2754–2774.

44. Lebrero, P.; Astudillo, A.M.; Rubio, J.M.; Fernández-Caballero, J.; Kokotos, G.; Balboa, M.A.; Balsinde, J. Cellular plasmalogen content does not influence arachidonic acid levels or distribution in macrophages: a role for cytosolic phospholipase A2γ in phospholipid remodeling. Cells 2019, 8, 799.
45. Axelsen, P.H.; Murphy, R.C. Quantitative analysis of phospholipids containing arachidonate and docosahexaenoate chains in microdissected regions of mouse brain. J. Lipid Res. 2010, 51, 660–671.
46. Fahy, E.; Subramaniam, S.; Brown, H.A.; Glass, C.K.; Merrill, A.H., Jr.; Murphy, R.C.; Raetz, C.R.; Russell, D.W.; Seyama, Y.; Shaw,W.; et al. A comprehensive classification system for lipids. J. Lipid Res. 2005, 46, 839–861.
47. Fahy, E.; Subramaniam, S.; Murphy, R.C.; Nishijima, M.; Raetz, C.R.; Shimizu, T.; Spener, F.; van Meer, G,; Wakelam, M.J.; Dennis. E.A. Update of the LIPID MAPS comprehensive classification system for lipids. J. Lipid Res. 2009 50, S9–S14.
48. Balgoma, D.; Astudillo, A.M.; Pérez-Chacón, G.; Montero, O.; Balboa, M.A.; Balsinde, J. Markers of monocyte activation re-vealed by lipidomic profiling of arachidonic acid-containing phospholipids. J. Immunol. 2010, 184, 3857–3865.
49. Gil-de-Gómez, L.; Astudillo, A.M.; Guijas, C.; Magrioti, V.; Kokotos, G.; Balboa, M.A.; Balsinde, J. Cytosolic group IVA and calcium-independent group VIA phospholipase A2s act on distinct phospholipid pools in zymosan-stimulated mouse perito-neal macrophages. J. Immunol. 2014, 192, 752–762.
50. Rubio, J.M.; Astudillo, A.M.; Casas, J.; Balboa, M.A.; Balsinde, J. Regulation of phagocytosis in macrophages by membrane ethanolamine plasmalogens. Front. Immunol. 2018, 9, 1723.
51. Rouzer, C.A.; Ivanova, P.T.; Byrne, M.O.; Milne, S.B.; Brown, H.A.; Marnett, L.J. Lipid profiling reveals glycerophospholipid remodeling in zymosan-stimulated macrophages. Biochemistry 2007, 46, 6026–6042.
52. MacDonald, J.I.; Sprecher, H. Phospholipid fatty acid remodeling in mammalian cells. Biochim. Biophys. Acta 1991, 1084, 105–121.
53. Chilton, F.H.; Fonteh, A.N.; Surette, M.E.; Triggiani, M.; Winkler, J.D. Control of arachidonate levels within inflammatory cells. Biochim. Biophys. Acta 1996, 1299, 1–15.
54. Yamashita, A.; Hayashi, Y.; Nemoto-Sasaki, Y.; Ito, M.; Oka, S.; Tanikawa, T.; Waku, K.; Sugiura, T. Acyltransferases and transacylases that determine the fatty acid composition of glycerolipids and the metabolism of bioactive lipid mediators in mammalian cells and model organisms. Prog. Lipid Res. 2014, 53, 18–81.
55. Humes, J.L.; Bonney, R.J.; Pelus, L.; Dahlgren, M.E.; Sadowski, S.J.; Kuehl, F.A.; Davies, P. Macrophages synthesise and re-lease prostaglandins in response to inflammatory stimuli. Nature 1977, 269, 149–151.
56. Scott, W.A.; Zrike, J.M.; Hamill, A.L.; Kempe, J.; Cohn, Z.A. Regulation of arachidonic acid metabolites in macrophages. J. Exp. Med. 1980, 152, 324–335.
57. Emilsson, A.; Sundler, R. Evidence for a catalytic role of phospholipase A in phorbol diester- and zymosan-induced mobiliza-tion of arachidonic acid in mouse peritoneal macrophages. Biochim. Biophys. Acta 1986, 876, 533–542.
58. Balsinde, J.; Fernández, B.; Solís-Herruzo, J.A.; Diez, E. Pathways for arachidonic acid mobilization in zymosan-stimulated mouse peritoneal macrophages. Biochim. Biophys. Acta 1992, 1136, 75–82.
59. Qiu, Z.H.; Leslie, C.C. Protein kinase C-dependent and -independent pathways of mitogen-activated protein kinase activa-tion in macrophages by stimuli that activate phospholipase A2. J. Biol Chem. 1994, 269, 19480–19487.
60. Satake, Y.; Diaz, B.L.; Balestrieri, B.; Lam, B.K.; Kanaoka, Y.; Grusby, M.J.; arm, J.P. Role of group V phospholipase A2 in zy-mosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. J. Biol. Chem. 2004, 279, 16488-16494.
61. Emilsson, A.; Sundler, R. Differential activation of phosphatidylinositol deacylation and a pathway via diphosphoinositide in macrophages responding to zymosan and ionophore A23187. J. Biol. Chem. 1984, 259, 3111–3116.

62. Hoffman, T.; Lizzio, E.F.; Suissa, J.; Rotrosen, D.; Sullivan, J.A.; Mandell, G.L.; Bonvini, E. Dual stimulation of phospholipase activity in human monocytes. Role of calcium-dependent and calcium-independent pathways in arachidonic acid release and eicosanoid formation. J. Immunol. 1988, 140, 3912–3918.
63. Balsinde, J.; Fernández, B.; Diez, E. Regulation of arachidonic acid release in mouse peritoneal macrophages. The role of ex-tracellular calcium and protein kinase C. J. Immunol. 1990, 144, 4298–4304.
64. Gijón, M.A.; Spencer, D.M.; Siddiqi, A.R.; Bonventre, J.V.; Leslie, C.C. Cytosolic phospholipase A2 is required for macrophage arachidonic acid release by agonists that do and do not mobilize calcium. J. Biol. Chem. 2000, 275, 20146–20156.
65. Shirai, Y.; Balsinde, J.; Dennis, E.A. Localization and functional interrelationships among cytosolic group IV, secreted group V, and Ca2+-independent group VI phospholipase A_2s in P388D1 macrophages using GFP/RFP constructs. Biochim. Biophys. Acta 2005, 1735, 119 –129.
66. Hartman, E.J.; Omura, S.; Laposata, M. Triacsin C: a differential inhibitor of arachidonoyl-CoA synthetase and nonspecific long chain acyl-CoA synthetase. Prostaglandins 1989, 37, 655–671.
67. Kim, J. H.,; Lewin, T.M.; Coleman, R.A.. Expression and characterization of recombinant rat acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. J. Biol. Chem. 2001; 276; 24667–24673.
68. Vessey, D.A.; Kelley, M.; Warren, R.S. Characterization of triacsin C inhibition of short-, medium-, and long-chain fatty acid: CoA ligases of human liver. J. Biochem. Mol. Toxicol. 2004, 18, 100–106.
69. Pérez-Chacón, G.; Astudillo, A.M.; Balgoma, D.; Balboa, M.A.; Balsinde, J. Control of free arachidonic acid levels by phos-pholipases A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 2009, 1791, 1103–1113.
70. Chilton, F.H.; Fonteh, A.N.; Sung, C.M.; Hickey, D.M.; Torphy, T.J.; Mayer, R.J.; Marshall, L.A.; Heravi, J.D.; Winkler, J.D. Inhibitors of CoA-independent transacylase block the movement of arachidonate into 1-ether-linked phospholipids of human neutrophils. *Biochemistry* 1995, 34, 5403–5410.
71. Winkler, J.D.; Fonteh, A.N.; Sung, C.M.; Heravi, J.D.; Nixon, A.B.; Chabot-Fletcher, M.; Griswold, D.E.; Marshall, L.A.; Chil-ton, F.H. Effects of CoA-independent transacylase inhibitors on the production of lipid inflammatory mediators. *J. Pharmacol. Exp. Ther.* 1995, 274, 1338–1347.
72. Ono, T.; Yamada, K.; Chikazawa, Y.; Ueno, M.; Nakamoto, S.; Okuno, T.; Seno, K. Characterization of a novel inhibitor of cytosolic phospholipase $A_2\alpha$, pyrrophenone. *Biochem. J*. 2002, 363, 727–735.
73. Ghomashchi, F.; Stewart, A.; Hefner, Y.; Ramanadham, S.; Turk, J.; Leslie, C.C.; Gelb, M.H. A pyrrolidine-based specific in-hibitor of cytosolic phospholipase A¬2α blocks arachidonic acid release in a variety of mammalian cells. *Biochim. Biophys. Acta* 2001, 1513, 160–166.
74. Flamand, N.; Picard, S.; Lemieux L.; Pouliot, M.; Bourgoin, S.G.; Borgeat, P. Effects of pyrrophenone, an inhibitor of group IVA phospholipase A_2, on eicosanoid and PAF biosynthesis in human neutrophils. *Br. J. Pharmacol*. 2006, 149, 385–392.
75. Degousee, N.; Ghomashchi, F.; Stefanski, E.; Singer, A.G.; Smart, B.P.; Borregaard, N.; Reithmeier, R.; Lindsay, T.F.; Lichten-berger, C.; Reinisch, W.; et al. Groups IV, V, and X phospholipases A_2s in human neutrophils: role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis. *J. Biol. Chem*. 2002, 277, 5061–5073.
76. Schevitz, R.W., Bach, N.J., Carlson, D.G., Chirgadze, N.Y.; Clawson, D.K.; Dillard, R.D.; Draheim, S.E.; Hartley, L.W.; Jones, N.D.; Mihelich, E.D., et al. Structure-based design of the first potent and selective inhibitor of human non-pancreatic secreto-ry phospholipase A_2. *Nat. Struct. Biol*. 1995, 2, 458-465.
77. Thommesen, L.; Sjursen, W.; Gåsvik, K.; Hanssen, W.; Brekke, O.L.; Skattebøl, L.; Holmeide, A.K.; Espevik, T.; Johansen, B.; Lægreid, A. Selective inhibitors of cytosolic or secretory phospholipase A_2 block TNF-induced activation of transcription factor nuclear factor-κB and expression of ICAM-1. *J. Immunol*. 1998, 161, 3421-3430.

78. Balsinde, J.; Balboa, M.A.; Yedgar, S.; Dennis, E.A. Group V phospholipase A_2-mediated oleic acid mobilization in lipopoly-saccharide-stimulated P388D1 macrophages. *J. Biol. Chem*. 2000, 275, 4783–4786.
79. Kokotos, G.; Hsu, Y.H.; Burke, J.E.; Baskakis, C.; Kokotos, C.G.; Magrioti, V.; Dennis, E.A. Potent and selective fluoroketone inhibitors of group VIA calcium-independent phospholipase A_2. *J. Med. Chem*. 2010, 53, 3602–3610.
80. Ali, T.; Kokotos, G.; Magrioti, V.; Bone, R.N,; Mobley, J.A.; Hancock, W.; Ramanadham, S. Characterization of FKGK18 as inhibitor of group VIA Ca^{2+}-independent phospholipase A_2 (iPLA$_2$β): candidate drug for preventing beta-cell apoptosis and diabetes. *PLoS One* 2013; 8: e71748.
81. Balsinde, J.; Balboa, M.A. Cellular regulation and proposed biological functions of group VIA calcium-independent phospho-lipase A_2 in activated cells. *Cell. Signal*. 2005, 17, 1052–1062.
82. Dennis, E.A.; Cao, J.; Hsu, Y.H.; Magrioti, V.; Kokotos, G. Phospholipase A_2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev*. 2011, 111, 6130–6185.
83. Ramanadham, S.; Ali, T.; Ashley, J.W.; Bone, R.N.; Hancock, W.D.; Lei, X. Calcium-independent phospholipases A_2 and their roles in biological processes and diseases. *J. Lipid Res*. 2015, 56, 1643–1668.
84. Arifin, S.A.; Falasca, M. Lysophosphatidylinositol signalling and metabolic diseases. *Metabolites* 2016, 6, 6.
85. Kita, Y.; Shindou, H.; Shimizu, T. Cytosolic phospholipase A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 2019, 1864, 838–845.
86. Chakravarthy, M.V.; Lodhi, I.J.; Yin, L.; Malapaka, R.R.; Xu, H.E.; Turk, J.; Semenkovich, C.F. Identification of a physiologi-cally relevant endogenous ligand for PPARα in liver. *Cell* 2009, 138, 476–488.
87. Lee, J.M.; Lee, Y.K.; Mamrosh, J.L.; Busby, S.A.; Griffin, P.R.; Pathak, M.C.; Ortlund, E.A.; Moore, D.D. A nucle-ar-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. *Nature* 2011, 474, 506–510.
88. O'Donnell, V.B; Murphy, R.C. New families of bioactive oxidized phospholipids generated by immune cells: identification and signaling actions. *Blood* 2012, 120, 1985–1992.
89. Casas, J.; Gijón, M.A.; Vigo, A.G.; Crespo, M.S.; Balsinde, J.; Balboa, M.A. Phosphatidylinositol 4,5-bisphosphate anchors cy-tosolic group IVA phospholipase A_2 to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* 2006, 17, 155–162.
90. Koeberle, A.; Shindou, H.; Harayama, T.; Shimizu, T. Palmitoleate is a mitogen, formed upon stimulation with growth fac-tors, and converted to palmitoleoyl-phosphatidylinositol. *J. Biol. Chem*. 2012, 287, 27244–27254.
91. Thürmer, M.; Gollowitzer, A.; Pein, H.; Neukirch, K.; Gelmez, E.; Waltl, L.; Wielsch, N.; Winkler, R.; Löser, K,; Grander, J.; et al. PI(18:1/18:1) is a SCD1-derived lipokine that limits stress signaling. *Nat. Commun*. 2022, 13, 2982.
92. Lands, W.E.M. Stories about acyl chains. *Biochim. Biophys. Acta* 2000, 1483, 1–14.
93. Murphy, R.C.; Folco, G. Lysophospholipid acyltransferases and leukotriene biosynthesis: Intersection of the Lands cycle and the arachidonate PI cycle. *J. Lipid Res*. 2019, 60, 219–226.
94. Mouchlis, V.D.; Chen, Y.; McCammon, J.A.; Dennis, E.A. Membrane allostery and unique hydrophobic sites promote enzyme substrate specificity. *J. Am. Chem. Soc*. 2018, 140, 3285–3291.
95. Hayashi, D.; Mouchlis, V.; Dennis, E.A. Each phospholipase A_2 type exhibits distinct selectivity toward sn-1 ester, alkyl ether, and vinyl ether phospholipids. *Biochim. Biophys. Acta* 2022, 1867, 159067.
96. Hayashi, D.; Mouchlis, V.; Dennis, E.A. Omega-3 versus omega-6 fatty acid availability is controlled by hydrophobic site geometries of phospholipase A_2s. *J. Lipid Res*. 2021, 62, 100113.
97. Murakami, M.; Masuda, S.; Ueda-Semmyo, K.; Yoda, E.; Kuwata, H.; Takanezawa, Y.; Aoki, J.; Arai, H.; Sumimoto, H.; Ishikawa, Y.; et al. Group VIB Ca^{2+}-independent phospholipase A_2β promotes cellular

membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A_2. *J. Biol. Chem*. 2005, 280, 14028–14041.
98. Deng, X.; Wang, J.; Jiao, L.; Utaipan, T.; Tuma-Kellner, S.; Schmitz, G.; Liebisch, G.; Stremmel, W.; Chamulitrat, W. $iPLA_2\beta$ deficiency attenuates obesity and hepatic steatosis in ob/ob mice through hepatic fatty-acyl phospholipid remodeling. *Biochim. Biophys. Acta* 2016, 1861, 449–461.
99. Jiao, L.; Gan-Schreier, H.; Zhu, X.; Wei, W.; Tuma-Kellner, S.; Liebisch, G.; Stremmel, W.; Chamulitrat. W. Ageing sensitized by $iPLA_2\beta$ deficiency induces liver fibrosis and intestinal atrophy involving suppression of homeostatic genes and alteration of intestinal lipids and bile acids. *Biochim Biophys Acta* 2017, 1862, 1520–1533.
100. Otto, A.C.; Gan-Schreier, H.; Zhu, X.; Tuma-Kellner, S.; Staffer, S.; Ganzha, A.; Liebisch, G.; Chamulitrat, W. Group VIA phospholipase A_2 deficiency in mice chronically fed with high-fat-diet attenuates hepatic steatosis by correcting a defect of phospholipid remodeling. *Biochim Biophys Acta* 2019, 1864, 662–676.
101. Zhu, X.; Gan-Schreier, H.; Otto, A.C.; Cheng, Y.; Staffer, S.; Tuma-Kellner, S.; Ganzha, A.; Liebisch, G.; Chamulitrat, W. $iPLA_2\beta$ deficiency in mice fed with MCD diet does not correct the defect of phospholipid remodeling but attenuates hepato-cellular injury via an inhibition of lipid uptake genes. *Biochim. Biophys. Acta* 2019, 1864, 677–687.
102. Astudillo, A.M.; Balboa, M.A.; Balsinde, J. Compartmentalized regulation of lipid signaling in oxidative stress and inflamma-tion: Plasmalogens, oxidized lipids and ferroptosis as new paradigms of bioactive lipid research. *Prog. Lipid Res*. 2023, 89, 101207.
103. Balboa, M.A.; Balsinde, J. Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem*. 2002, 277, 40384–40389.
104. Balsinde, J.; Dennis, E.A. Distinct roles in signal transduction for each of the phospholipase A_2 enzymes present in $P388D_1$ macrophages. *J. Biol. Chem*. 1996, 271, 6758–6765.
105. Pindado, J.; Balsinde, J.; Balboa, M.A. TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A_2/cyclooxygenase-2 pathway. *J. Immunol*. 2007, 179, 4821–4828.
106. Diez, E.; Balsinde, J.; Aracil, M.; Schüller, A. Ethanol induces release of arachidonic acid but not synthesis of eicosanoids in mouse peritoneal macrophages. *Biochim. Biophys. Acta* 1987, 921, 82–89.
107. Balsinde, J.; Diez, E.; Mollinedo, F. Phosphatidylinositol-specific phospholipase D: a pathway for generation of a second messenger. *Biochem. Biophys. Res. Commun*. 1988, 154, 502–508.
108. Balsinde, J.; Diez, E.; Fernández, B.; Mollinedo, F. Biochemical characterization of phospholipase D activity from human neutrophils. *Eur. J. Biochem*. 1989, 186, 717–724.
109. Balboa, M.A.; Balsinde, J.; Dennis, E.A.; Insel, P.A. A phospholipase D-mediated pathway for generating diacylglycerol in nuclei from Madin-Darby canine kidney cells. *J. Biol. Chem*. 1995, 270, 11738–11740.
110. Johnson, C.A.; Balboa, M.A.; Balsinde, J.; Dennis, E.A. Regulation of cyclooxygenase-2 expression by phosphatidate phosphohydrolase in human amnionic WISH cells. *J. Biol. Chem*. 1999, 274, 27689–27693.
111. Balboa, M.A.; Balsinde, J.; Johnson, C.A.; Dennis, E.A. Regulation of arachidonic acid mobilization in lipopolysaccharide-activated $P388D_1$ macrophages by adenosine triphosphate. *J. Biol. Chem.* 1999, 274, 36764–3768.
112. Balboa, M.A.; Balsinde, J.; Dennis, E.A. Phosphorylation of cytosolic group IV phospholipase A_2 is necessary but not sufficient for arachidonic acid release in $P388D_1$ macrophages. *Biochem. Biophys. Res. Commun*. 2000, 267, 145–148.
113. Gil-de-Gómez, L.; Astudillo, A.M.; Lebrero, P.; Balboa, M.A.; Balsinde, J. Essential role for ethanolamine plasmalogen hydrolysis in bacterial lipopolysaccharide priming of macrophages for enhanced arachidonic acid release. *Front. Immunol*. 2017, 8: 1251.

114. Rodríguez, J.P.; Guijas, C.; Astudillo, A.M.; Rubio, J.M.; Balboa, M.A.; Balsinde, J. Sequestration of 9-hydroxystearic acid in FAHFA (fatty acid esters of hydroxy fatty acids) as a protective mechanism for colon carcinoma cells to avoid apoptotic cell death. *Cancers* 2019, 11, 524.
115. Astudillo, A.M.; Balgoma, D.; Balboa, M.A.; Balsinde, J. Dynamics of arachidonic acid mobilization by inflammatory cells. *Biochim. Biophys. Acta* 2012, 1821, 249–256.
116. Astudillo, A.M.; Balboa, M.A.; Balsinde, J. Selectivity of phospholipid hydrolysis by phospholipase A_2 enzymes in activated cells leading to polyunsaturated fatty acid mobilization. *Biochim. Biophys. Acta* 2019, 1864, 772–783.

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