Lipids: Role in Embryonic Development (Review)

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Authors' contributions

This work was carried out in collaboration among all authors. Author ML designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors ACBR, AOMT, LFM, LMD and ALJD managed the literature searches, organized and wrote the article. Author ADC designed the images, and helped in the writing and revision of the article. Author DEL translated, revised and rewrote part of the article. Author MAM advisor and work design. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Aims: We propose to briefly review the specific role of lipids in embryonic structures development.
Results: Lipids are organic substances insoluble in water, divided into several classes, such as fatty acids, glycolipids, phospholipids, ceramides, sphingolipids, and stereo-lipids. They participate in processes of cellular metabolism and embryonic development which are associated with signalling, proliferation and cell migration. They act in developmental processes such as calcification and bone mineralization, pulmonary maturity, cellular differentiation, and neural survival, epithelial cells polarization and muscle formation, in which phospholipids as a major group, work more regularly. Lipids during embryonic development work directly as transport molecules or cell markers. In addition to an imbalance in its enzymatic and protein precursors (such as choline kinase), lipids can increase or decrease lipid concentration in cells, prevent its biotransformation, or affect its synergy with other molecules, leading to failures in the formation of organs such as the heart, brain, and bones. This aims to further the understanding of these processes and highlight its feasibility for future clinical applications.

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Conclusion: Lipids maintain cell membrane integrity in blastocysts, transport calcium to nerve and bone cells, facilitate neural apoptosis, and promote pulmonary maturation. These results aid in the understanding and prediction of alterations in lipidic metabolic syndromes in several pathological disorders during organ development.

Keywords: *Lipids; embryonic development; embryonic lethality; pulmonary maturation; neural development; bone formation.*

1. INTRODUCTION

Lipids are organic substances insoluble in water, divided into several classes, and the most studied are fatty acids, glycolipids, phospholipids, sphingolipids, and stereo-lipids [1,2]. Mammalian cells contain more than 1000 different types of lipids, but phospholipids are the ones with the largest number of molecules distributed throughout the cell [3]. This group is subdivided into four subgroups: those containing choline, such as phosphatidylcholine (PC) and sphingomyelin (SM), and those containing amino phospholipids, such as phosphatidylethanolamine (PE) and phosphatidylserine (PS) [1]. Lipids are distributed in cell membranes and in organelles such as mitochondria and Golgi complex, to perform processes such as synthesis, degradation, migration and intercellular communication [4].

During the embryogenesis they play an important role maintaining functions as mediators of formation and transport in cellular membranes, they also participate as signposts and activators of migration, proliferation, differentiation and cell adhesion [2]. Besides that, lipids also prevent developmental defects in the organism, as they facilitate the transport of nutrients, function as an energy source and construct organs such as brain and muscles [5].

Due to its importance during the process of embryo formation, the study has the aim to review the role of different lipid classes in embryonic development, emphasizing their participation in systemic development.

2. LIPIDS: LETHALITY AND EMBRYONIC VITALITY

Lipids role in organogenesis is directly related with substance captation and with cell signalling; or indirectly when they become unbalanced by alterations in their biotransformative proteins, or when they join to protein cascades, which can lead to congenital malformations or to produce embryonic lethality [6]. An example of these proteins is choline kinase α (CK- α), an essential

biotransformation protein in the synthesis of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) (essential cell membrane lipids) [7], the lack of CK- α causes imbalance in the cell membrane due to deficiency of PC, PE and PS. When this imbalance occurs in the cell membranes of blastocysts, early embryonic lethality occurs due to the inability of the self-regulating embryo [8], [9]. This mechanism alters the phospholipid biosynthetic pathways, such as lack of CK- α , phosphocholine cytidyl transferase (CTP) and phosphatidylserine decarboxylase, can produce embryonic lethality in mice [10] (Table 1 shows a summary of lipids pathways in embryonic development).

On one hand, early absence of CTP in mice, a catalyst that provides the biosynthesis rate of phosphocholine and phosphatidylethanolamine, inhibits zygote transformation into a blastocyst, preventing cell proliferation and embryo implantation, which may cause early lethality. When this imbalance occurs in fetuses, tissue enzymatic activity is reduced by 50% [11].

On the other hand, phosphatidylserine decarboxylase is essential for the synthesis of PE in mitochondria [12]. Steenbergen et al. showed that the alteration of this process in mutant mice reduces the production of PE, which causes fragmentation and mitochondrial deformity, which increases the lethality of embryos between days 8 and 10 of pregnancy [13].

Another case of indirect action is in the deficiency of glucose-6-phosphate dehydrogenase (G6PD) a human hereditary disease, in which there is increased activity of phospholipase A2 (iPLA), with a decrease in nicotinamide adenine dinucleotide phosphate (NADPH) that together with the high peroxidation of lipids and an increase of lysoglycerophospholipids, cause cell membrane permeability defects, polarization and cytokinesis, producing early embryo lethality in murine models and in *Caenorhabditis elegans* [14,15].

Table 1. Summary of the role of lipids in the cellular metabolism of development.

Role	Lipids	Action
Embryonic Lethality	PC; PE; PS; PI; FFA	Destabilize cellular membrane. Interrupt morphogenesis [8], [16]–[11]
	Ceramides	Provoke aberrant proliferation and differentiation [17]
	PE	Deform mitochondria [12], [13]
	FFA, PI	Cause exocytosis and cytokinesis [18]
Bone Mineralization	TR; FFA, Cholesterol;	Promote cellular growth and proliferation [19], [20]
	PS, TR, FFA, Cholesterol	Activate intracellular signalling [21]
	TR; SM; PS; PI; Cholesterol	Form lipid vacuoles in the bone matrix [21]–[24]
Formation of the nervous system.	PI	Causes neural apoptosis [25]
	PI; SM	Form synapses and neuroexocitosis [26]
	PI; Cholesterol	Promote cell proliferation and migration [27]–[29]
Pulmonary development.	PG; PI	Foster pulmonary maturation. Produce pulmonary surfactant [6], [30], [31]
Kidneys	PI	Stimulate cell migration. Establish epithelial cell polarity [10]
Muscle formation	Ceramides	Affect metabolism by mitochondrial disorders [3], [17]

PC (Phosphatidylcholine); PE (Phosphatidylethanolamine); PS (Phosphatidylserine); PG (Phosphoglycerides); Ceramides (CR); TR (Triglycerides); SM (Sphingolipids); FFA (Free Fatty Acids); PI (Phosphatidylinositol).

Fig. 1. Anchoring the glycosylphosphatidylinositol in the cell membrane.

Glycosylphosphatidylinositol (GPI) is a post-transductional modification that results in the binding of modified proteins to the outer membrane of the plasma membrane. Tissue culture experiments have shown that GPI-anchored proteins (GPI-AP) reach the apical membrane of epithelial cells [Authors]

Also, alterations in free fatty acids (FFA) esterification, by an enzymatic imbalance in acyl-CoA synthesis (ACS), indirectly affects maternal lipids availability, signal transduction and the

embryo development (zygotes), producing exocytosis and cytokinesis that lead to early embryonic lethality in humans [32].

Moreover, faulty ceramides transport in the absence of ceramide transferase (in live mice) can stress the endoplasmic reticulum and that describes this effect the development and embryonic growth, causing a decrease of cell proliferation and aberrant differentiation, resulting in imminent embryo lethality due to excessive cell death [17]. Lipids such as phospholipids (specifically phosphoethanolamine) act in the uptake of substances such as glycosylphosphatidylinositol (GPI) (Fig. 1), anchoring structures to the cell membrane [33]. Alterations or mutations in the functionality of the GPI in mice, interrupt the stretching phase of morphogenesis and/or ruptures in epidermal cells membrane, which leads to early embryonic lethality. This suggests that the cortex connection to the epithelial membrane is weakening as is observed in multiple congenital malformation syndrome and congenital renal abnormalities in humans (9).

Another important subgroup is the phosphoinositides (PI), which combined to actin-myosin, provide the plasma membrane expansion and contraction in *Caenorhabditis elegans* embryo, which activate cell differentiation [18,34,35]. Increased PI levels lead to premature contraction and cellular shortening. Moreover, it produces expansion and premature cellular contractility, varying the cellular form during morphogenesis and leading to embryonic lethality [36].

3. LIPIDS: MINERALIZATION AND BONE FORMATION

The mineralization and calcification of bones and cartilages are essential for skeleton development at the end of gestation. Since 1974, lipids have been related to calcification and bone mineralization, through phospholipids, cholesterol, and glycerol identification in epiphyseal cartilage development [22,23]. Other lipid structures were identified as small vesicles rich in sphingomyelin, phosphatidylserine, and cholesterol [21] (summarized in table 1).

Mesenchymal stem cells derived from adipose tissue (ASCs) has been shown by Elashry et al. to improve calcification, cellular proliferation, and differentiation of cell in equine models of osteopenia [37]. Similarly, human ASCs cells (hASC) have been used in bone tissue

bioengineering [37-39], as they were shown to be rich in lipid vacuoles responsible for transporting triglycerides in Golgi Complex [19], [40], as well as nuclear lipids such as fatty acids and cholesterol [20], who play a key role in the handling and transport in the cell membrane. These cells have a high osteogenic differentiation potential with protein kinase activation and extracellular signal inhibition, which produces osteogenesis and calcium elimination [41].

In bone formation, [21] it was demonstrated that the composition of lipids in bones would be distributed in cell membrane (non-polar lipids, mainly sphingomyelin, phosphoglycerides, triglycerides, cholesterol esters, free cholesterol, and fatty acids) and extracellular vacuoles (greater amount of phospholipids and cholesterol) acting directly in bone adjustment. Growth and cell proliferation in bones initiate with intracellular signals activation (Fig. 2), which activate the kinase to generate phospholipid metabolites. This process begins with lipid vacuoles formation in bone osteoid within the newly formed bone and with calcium release produced by the action of phospholipase C- γ 1 (PLC- γ 1) (the enzyme responsible for the hydrolysis of phosphatidylinositol 4.5 bisphosphate), which releases calcium from the endoplasmic reticulum to produce amorphous calcium phosphate [24]. For mineralization to occur, a force is required to transport calcium and other minerals to the ossification centre in the bone, composed of calcium-lithium phosphate complex (CPLX - amorphous calcium phosphate and phosphatidylserine) and annexin A5 (a protein and dependent on Ca^{2+} lipids) [42]. Following, crystal accumulates in the area and maintains the skeleton integrity, strengthening, and bone rotation [43].

CK protein-related diseases indirectly reduce PC synthesis and participate in the Kennedy route that catalyzes choline-CDP for PC synthesis, that has an important role in embryonic lethality [7,44]. CK inhibition and deficiency in PC synthesis have been related to abnormal development of bones in peacocks and bone remodelling in rats [45]. In addition, an alteration of CDP-choline route, decline 80% of CP, forming extensive degradation of cell membrane, leading to a decrease in proliferation and differentiation of chondrocytes, which consequently causes deformities in forearm bones with expanded hypertrophic zones, as well as, proliferation and erroneous growth plates formation and ossification centers [46].

Fig. 2. The role of lipids in bone calcification. Calcification and mineralization initiate in the dialysis of long bones. It begins with the formation of lipid vesicles in the bone matrix (osteoid), followed by the release of calcium (CA) from the endoplasmic reticulum by the action of phospholipase C-γ1 (PLC-γ1) in 1. Free calcium in cellular plasma, by the action of a driving force to a nucleus of ossification and Annexin A5 in 2. Following, there is an accumulation of crystals, production in the bone matrix [Authors].

4. LIPIDS: EMBRYONIC DEVELOPMENT OF THE NERVOUS SYSTEM

In animals, during nervous system development, neural tube development is conducted with lipids participation, avoiding failures during the process (Table 1) [27]. Furthermore, phosphatidylinositol collaborates with GPI, functioning as a molecular receptor for epinephrine on the cell surface. A failure of this mechanism at neurulation in mice would cause inhibition of spinal neural closure, with defects in neural development, without adverse effects on the growth or progression of embryonic development [28].

In morphogenesis, neuronal studies showed that the phospholipid phosphatidylinositol 3,4-bisphosphate (PI (3,4) P2) group is required for neurogenesis programming, they act directly to determine the onset of neurite (necessary for neural circuit beginning) [47]. Under other conditions, phosphatidylinositol participates

indirectly in transduction of neurotransmitters and hormones, starting from phospholipase C, a bio transforming enzyme present in neurons, astrocytes, and oligodendrocytes [26], which can affect neural formation since it is an activator of inositol phosphatidylinositol phosphate and glycerol that, with phosphatidylinositol-3-phosphate, activates the neuroexocytosis process [48].

In nervous system development, approximately half of the neurons generated enter apoptosis, only those with correctly formed and functional synapses remain active. This process of apoptosis is regulated by a signalling cascade that includes insulin-like growth factor 1 (IGF-1) and PI, which acts as a synaptic receptor present in the cell body and synaptic clefts [25]. This process is also regulated by the phosphoinositide-dependent protein kinase 1 (PDK1) that inhibits neuron apoptosis. Phosphoinositide 3-kinase connects with

phosphatidylinositol-3,4,5-triphosphate, activates a kinase chain family activating PDK1 by interrupting its interaction with phosphoinositide, and then PDK1 regulates neural apoptosis. Other studies, showed that ceramides and its derivative, sphingosine-1-phosphate (S1P) are fundamental for differentiation of neural stem cells and apoptosis, which also perform differentiation of embryonic cells [49].

The brain of embryos and fetuses, as well as adults, synthesizes cholesterol locally. Although this mechanism is still a process under study, it is believed that cortical neurons need an indispensable biosynthesis of cholesterol for their maintenance and survival, thus acting directly in maintenance of the developing brain. The brain development needs a higher demand for cholesterol to keep neurites growth, maintain the number of functional axons and neuronal differentiation. These neurons only tolerate a slight alteration in cholesterol biosynthesis, which may have neurodegenerative implications [29]. Due to the presence of cholesterolemia enzymes, cholesterol has been identified in mice embryos in neural crest derivatives, neural tube, brain, and extremities [50,51].

Brain lipids, such as sphingomyelin, GM1 ganglioside, and sphingolipid derivatives, have a direct cell signalling function (bioactive lipids). These act by associations or protein binding, lipid rafts formation that regulates cellular signalling protein and messengers activity in the energy balance for differentiation of neural stem cells (NSC) [52]. A review carried out by Ryoul et al. [26] describes how gangliosides participate in early differentiation and embryonic cerebral maturation, cerebral synapses and neurites establishment.

5. LIPIDS: LUNG MATURATION

Glycerides and phosphoglycerides are related in mammals respiratory system development, mainly in lung maturation (Table 1) [6,31]. Lung maturation is correlated to the presence of pulmonary surfactant, a substance present on lung alveolar epithelial surface that prevents its collapse upon expiration [30]. This protective constituent contains phospholipids, mainly phosphatidylcholine, which is formed at the end of gestation, increasing its synthesis with the body development progress [53].

Phosphatidylcholine (PC) is the main phospholipid of the cellular membrane in most

animals, accounting for about half of the phospholipids in cells and more than half of serum phospholipids. It has a structural role in cell membranes, where it forms the matrix of the bilayer. In addition to being an essential component of pulmonary surfactant, it helps to transport cholesterol through the body [54].

In addition to phosphatidylcholine, pulmonary surfactant is also rich in phosphatidylglycerol, another surfactant component, as well as enzymes involved in the synthesis of phosphatidylcholine and phosphatidylglycerol, such as phosphocholine transferase [53]. In fetal lung, the percentage of phosphatidylinositol increases concomitantly with phosphatidylcholine, followed by phosphatidylglycerol [30].

The production of these compounds is carried out by three mechanisms: by increase of microsomal glycerol phosphate that activates phosphatidyltransferase and reduces the increase of phosphatidylglycerol, serum inositol decrease at the end of gestation, which produces a reduction in phosphatidylinositol and an increase in phosphatidylglycerol; also synthesis of phosphatidylglycerol is activated by the increase of phosphatidylcholine [30].

6. CONCLUSION

Lipids have been studied mostly through their molecular mediators by bio transforming and synthesizing enzymes such as phosphocholine, cytidyltransferase, and phospholipase C, or precursor proteins such as choline kinase. Each specific group of lipids has an important role in embryonic development acting as signalling agents, membrane receptor, and transporters.

Since lipids are linked to maintaining integrity of cell membrane in blastocysts, transport of calcium to nerve and bone cells, neural apoptosis, and in pulmonary maturation, it is expected that, in extended time, these results can be used to understand and foresee alterations in metabolic syndromes of lipids in several pathological disorders through organ development.

CONSENT

All the authors declare that a literature review article was made, the concentration of patients was not necessary, and that all the bibliographic data used are duly cited.

ETHICAL APPROVAL

All authors declare that there have been no experiments on animals or humans.

COMPETING INTERESTS

We inform that all the authors contributed equally to the execution of this manuscript, and it has not been sent to any other journal. All the authors approved the presentation, and do not have conflicts of interest.

REFERENCES

- Mateos G, Rebollar P, Medel P. Utilización de grasas y productos lipídicos en alimentación animal: Grasas puras y mezclas. *Mundo Veterinario*. 2000;4(2): 78–88.
- Park H, Haynes CA, Nairn AV, Kulik M, Dalton S, Moremen K, Merrill AH. Transcript profiling and lipidomic analysis of ceramide subspecies in mouse embryonic stem cells and embryoid bodies. *J Lipid Res*. 2010;51(3):480–9.
- Sher RB, Aoyama C, Huebsch KA, Ji S, Kerner J, Yang Y, Frankel WN, Hoppel CL, Wood PA, Vance DE, Cox GA. A rostrocaudal muscular dystrophy caused by a defect in choline kinase beta, the first enzyme in phosphatidylcholine biosynthesis. *J Biol Chem*. 2006;281(8): 4938–48.
- Kainu V, Hermansson M, Somerharju P. Electrospray ionization mass spectrometry and exogenous heavy isotope-labeled lipid species provide detailed information on aminophospholipid acyl chain remodeling. *J Biol Chem*. 2008;283(6):3676–87.
- Sudano MJ, Rascado TDS, Tata A, Belaz KRA, Santos VG, Valente RS, Mesquita FS, Ferreira CR, Araujo JP, Eberlin MN, Landim-Alvarenga FD. Lipidome signatures in early bovine embryo development. *Theriogenology*. 2016;86(2): 472–84.
- Hallman M, Slivka S, Wozniak P, Sills J. Perinatal Development of Myoinositol Uptake into Lung Cells: Surfactant Phosphatidylglycerol and Phosphatidylinositol. *Synth Rabbit*. 2002; 20(2):179–185.
- Aoyama C, Liao H, Ishidate K. Structure and function of choline kinase isoforms in mammalian cells. *Prog Lipid Res*. 2004; 43(3):266–81.
- Wu G, Aoyama C, Young SG, Vance DE. Early embryonic lethality caused by disruption of the gene for choline kinase α , the first enzyme in phosphatidylcholine biosynthesis. *J Biol Chem*. 2008;283(3): 1456–62.
- Wu G, Sher RB, Cox GA, Vance DE. Biochimica et biophysica acta differential expression of choline kinase isoforms in skeletal muscle explains the phenotypic variability in the rostrocaudal muscular dystrophy mouse. *Biochim Biophys Acta*. 2010;1801(4):446–54.
- Pinal N, Goberdhan DCI, Collinson L, Fujita Y, Cox IM, Wilson C, Pichaud F. Regulated and polarized PtdIns(3,4,5)P₃ accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol*. 2006;16(2):140–9.
- Wang L, Magdaleno S, Tabas I, Jackowski S. Early embryonic lethality in mice with targeted deletion of the CTP: phosphocholine cytidyltransferase α gene (Pcyt1a). *Mol Cell Biol*. 2005;25(8):3357–63.
- Kojima R, Endo T, Tamura Y. A phospholipid transfer function of ER-mitochondria encounter structure revealed in vitro. *Sci Rep*. 2016;6:30777.
- Steenbergen R, Nanowski TS, Beigneux A, Kulinski A, Young SG, Vance JE. Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. *J Biol Chem*. 2005;280(48): 40032–40.
- Chen T, Yang H, Hung C, Ou M, Pan Y, Cheng M, Stern A, Lo SJ, Chiu DT. Impaired embryonic development in glucose-6-phosphate dehydrogenase-deficient *Caenorhabditis elegans* due to abnormal redox homeostasis induced activation of calcium-independent phospholipase and alteration of glycerophospholipid metabolism. *Cell Death Dis*. 2017;8(1):e2545.
- Yang H, Chen T, Wu Y, Cheng K, Lin Y, Cheng M, Ho HY, Lo SJ, Chiu DT. Glucose 6-phosphate dehydrogenase deficiency enhances germ cell apoptosis and causes defective embryogenesis in *Caenorhabditis elegans*. *Cell Death Dis*. 2013;4:e616.
- Budirahardja Y, Doan TD, Zaidel-bar R. Glycosyl Phosphatidylinositol Anchor Biosynthesis Is Essential for Maintaining

- Epithelial Integrity during *Caenorhabditis elegans* Embryogenesis. *PloS Genet.* 2015; 11(3):e1005082.
17. Wang X, Rao RP, Kosakowska-cholody T, Masood MA, Southon E, Zhang H, Berthet C, Nagashim K, Veenstra TK, Tessarollo L, Acharya U, Acharya JK. Mitochondrial degeneration and not apoptosis is the primary cause of embryonic lethality in ceramide transfer protein mutant mice. *J Cell Biol.* 2009;184(1):143–58.
18. Figarar L, Xu H, Garcia HG, Golding I, Sokac AM. The plasma membrane flattens out to fuel cell surface growth during *Drosophila* cellularization. *Dev Cell.* 2013; 27(6):648–55.
19. Hesse D, Jaschke A, Chung B, Schurmann A. Trans -Golgi proteins participate in the control of lipid droplet and chylomicron formation. *Biosci Rep.* 2013;33(1):1-10.
20. Layerenza JP, González P, Bravo MMG De, Polo MP, Sisti MS, Ves-losada A. Biochimica et Biophysica Acta Nuclear lipid droplets: A novel nuclear domain. *BBA - Mol Cell Biol Lipids.* 2013;1831(2):327–40.
21. Wuthier RE. Lipid composition of isolated epiphyseal cartilage cells, membranes and matrix vesicles. *Biochim Biophys Acta.* 1975;409(1):128-43.
22. Peress NS, Anderson HC, Sajdera SW. The lipids of matrix vesicles from bovine fetal epiphyseal cartilage. *Calcif Tissue Res.* 1974;14(1):275-81.
23. Merolli A, Santin M. Role of phosphatidylserine in bone repair and its technological exploitation. *Molecules.* 2009;14(12):5367-81.
24. Crooke CE, Pozzi A, Carpenter GF. PLC- γ 1 regulates fibronectin assembly and cell aggregation. *Exp Cell Res.* 2009;315(13): 2207–14.
25. Brunet A, Datta SR, Greenberg ME. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr Opin Neurobiol.* 2001;11(3):297-305.
26. Ryoul Y, Kang D, Lee C, Seok H, Follo MY, Cocco L, Suh PG. Primary phospholipase C and brain disorders. *Adv Biol Regul.* 2015;61:80-50.
27. Zurashvili T, Cordon-Barris L, Ruiz-Babot G, Zhou X, Lizcano JM, Gomez N, Gimenez-Llort L, Bayascas JR. Interaction of PDK1 with Phosphoinositides Is Essential for Neuronal Differentiation but Dispensable for Neuronal Survival. *Mol Cell Biol.* 2013;33(5):1027–40.
28. Noraishah AM, Turmaine M, Greene NDE, Copp AJ. EphrinA-EphA receptor interactions in mouse spinal neurulation: implications for neural fold fusion. 2009; 568:559–68.
29. Funkschilling U, Jockusch WJ, Sivakumar N, Möbius W, Corthals K, Sai Li, Quintes S, Kim Y, Schaap IA, Rhee JS, Nave KA, Saher G. Critical time window of neuronal cholesterol synthesis during neurite outgrowth. *J Neurosci.* 2012;32(22):7632-45.
30. Batenburg JJ, Klazinga W, Van Golde LM. Regulation of phosphatidylglycerol and phosphatidylinositol synthesis in alveolar type II cells isolated from adult rat lung. *FEBS Lett.* 1982;147(2):171–4.
31. Bleasdale JE, Maberry MC, Quirk JG. Myo-inositol homeostasis in foetal rabbit lung. *Biochem J.* 1982;206(1):43–52.
32. Kniazeva M, Shen H, Euler T, Wang C, Han M. Regulation of maternal phospholipid composition and IP 3 -dependent embryonic membrane dynamics by a specific fatty acid metabolic event in *C. elegans*. *Genes Dev.* 2012; 26(6):554-66.
33. Munday AD, López JA. Posttranslational protein palmitoylation: Promoting platelet purpose. *Arterioscler Thromb Vasc Biol.* 2007;27(7):1496–9.
34. Comer FI, Parent CA. Phosphoinositides specify polarity during epithelial organ development. *Cell.* 2007;128(2):239-40
35. Janetopoulos C, Devreotes P. Phosphoinositide signaling plays a key role in cytokinesis. *J Cell Biol.* 2006;174(4): 485-90.
36. Reversi A, Loeser E, Subramanian D, Schultz C, Renzis S De. Plasma membrane phosphoinositide balance regulates cell shape during *Drosophila* embryo morphogenesis. *J Cell Biol.* 2014; 205(3):395-408.
37. Elashry MI, Baulig N, Heimann M, Bernhardt C, Wenisch S, Arnhold S. Research in Veterinary Science Osteogenic differentiation of equine adipose tissue derived mesenchymal stem cells using CaCl 2. *Res Vet Sci.* 2018;117: 45-53.
38. Dufrane D. Impact of age on human adipose stem cells for bone tissue engineering. 2017;26(9):1496–504.
39. Lee Y, Lee S, Cheol S, Sung J, Kadam AA. Surface functionalization of halloysite nanotubes with supermagnetic iron oxide,

- chitosan and 2-D calcium-phosphate nano flakes for synergistic osteoconduction enhancement of human adipose tissue-derived mesenchymal stem cells. *Colloids Surfaces B Biointerfaces*. 2019;173:18-26.
40. Gojanovich AD, Gimenez MC, Masone D, Rodriguez TM, Dani C. Human adipose-derived mesenchymal stem / stromal cells handling protocols. *Lipid Droplets and Proteins Double-Staining*. *Front Cell Dev Biol*. 2018;6:331-16.
 41. Liu Q, Cen L, Zhou H, Yin S, Liu G, Liu W, Cui LN. Extracellular signal-related kinase signaling pathway in osteogenic differentiation of human adipose-derived stem cells and in adipogenic transition initiated by dexamethasone. *O Title. Tissue Eng Part A*. 2009;15(11):3487-3497.
 42. Genge BR, Wu LNY, Wuthier RE. Kinetic analysis of mineral formation during in vitro modeling of matrix vesicle mineralization: Effect of annexin A5, phosphatidylserine, and type II collagen. *Anal Biochem*. 2007; 367(2):159–66.
 43. Houston B, Stewart AJ, Farquharson C. PHOSPHO1 - A novel phosphatase specifically expressed at sites of mineralisation in bone and cartilage. *Bone*. 2004;34(4):629-37.
 44. Eberlin LS, Ifa DR, Wu C, Cooks RG. Three-dimensional visualization of mouse brain by lipid analysis using ambient ionization mass spectrometry. *Angew Chem Int Ed Engl*. 2010;49(5):873-6.
 45. Gallego-Ortega D, Gómez del Pulgar T, Valdés-Mora F, Cebrián A, Lacal JC. Involvement of human choline kinase alpha and beta in carcinogenesis: A different role in lipid metabolism and biological functions. *Adv Enzyme Regul*. 2011;51(1):183–94.
 46. Li Z, Wu G, Van Der Veen JN, Hermansson M, Vance DE. Phosphatidylcholine metabolism and choline kinase in human osteoblasts. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2014;1841(6):859–67.
 47. Zhang S, Duan L, He S, Zhuang G, Yu X. Phosphatidylinositol 3,4-bisphosphate regulates neurite initiation and dendrite morphogenesis via actin aggregation. *Cell Res*. 2017;27(2):243-73.
 48. Mizuguchi M, Yamada M, SUK I, Rhee SG. Phospholipase C isozymes in neurons and glial cells in culture: an immunocytochemical and immunochemical study. *Brain Res*. 1991;548:35-40.
 49. Bieberich E. There is more to a lipid than just being a fat: sphingolipid-guided differentiation of oligodendroglial lineage from embryonic stem cells. *Neurochem Res*. 2011;36(9):1601-1611.
 50. Laubner D, Breitling R, Adamski J. Embryonic expression of cholesterologenic genes is restricted to distinct domains and colocalizes with apoptotic regions in mice. *Brain Res Mol Brain Res*. 2003;115(1):87-92.
 51. Roux C, Wolf C, Mulliez N, Gaoua W, Cormier V, Chevy F, Citadelle D. Role of cholesterol in embryonic development 1 – 4. 2018;71:1270–9.
 52. Bieberich E. It's a lipid's world: Bioactive lipid metabolism and signaling in neural stem cell differentiation. *Neurochem Res*. 2012;37(6):1208-29.
 53. Rooney, S. Gobran, L. Marino, A. Maniscalco, W. Gross I. Effects of betamethasone on phospholipid content, composition and biosynthesis in the fetal rabbit lung. *Biochim Biophys Acta*. 1979; 572(2):64-76.
 54. Kent C. Regulation of Phosphatidylcholine: Choline. *Prog Lipid Res*. 1991;29:87-105.

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