
# Essential phospholipids – mechanism of action in liver disease explained

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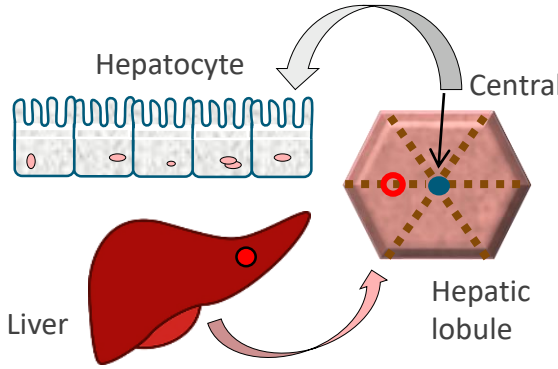
# Learning objectives

1. Understand the roles of phospholipids in hepatocytes and the key characteristics and composition of EPL
2. Review the pre-clinical evidence for EPL in liver disease and understand their pleiotropic mechanisms of action
3. Describe clinical evidence that supports the use of EPL in the management of patients with fatty liver disease with and without type 2 diabetes

EPL, essential phospholipids

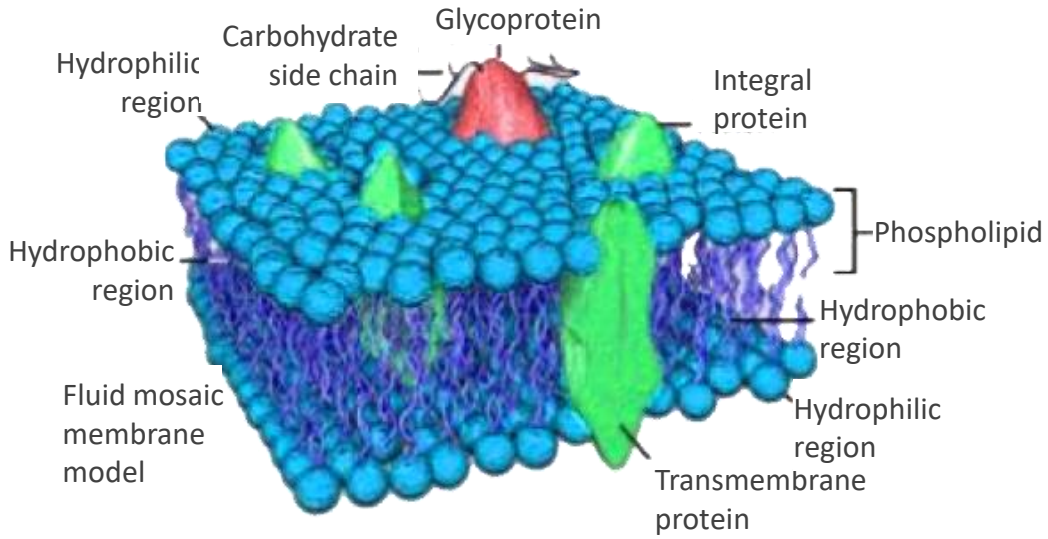
# Phospholipids in the human liver

- The human liver is composed of **multiple hepatic lobules consisting of hepatocytes**[1]
- Hepatocyte membranes are made up primarily of a **bilayer of phospholipids** affecting their fluidity, integrity, and function[1,2]
- Phosphatidylcholine (PC) constitutes one of the most abundant forms of phospholipids representing the building block for cell membranes (approximately 65% PC)[2,3,4]




*Adapted from: Smith ME& Morton DG. Chapter 6. Liver and biliary system. In: The digestive system, Second edition. 2010.*

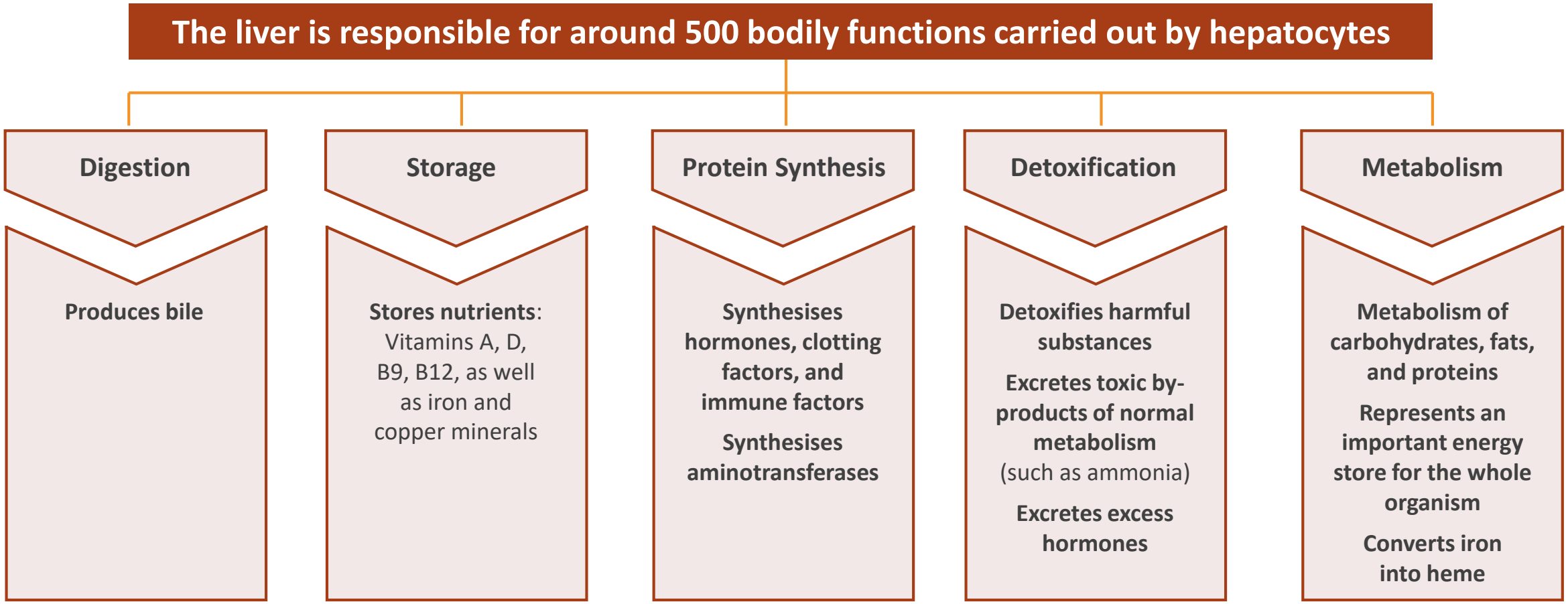
**Plasma membrane structural components**[5]




PL, phospholipid; PPC, phosphatidylcholine

1. Anatomy and physiology of the liver. Available from: https://web.unicz.it/uploads/2018/03/fegato.pdf; 2. Hussein JS. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5:38–46;
3. Gundermann KJ, et al. Pharmacol Rep 2011;63(3):643–59; 4. Kidd P. Altern Med Rev 1996;1(4):258–74; 5. Adapted from Hussein JS. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5:38–46

# Liver functions



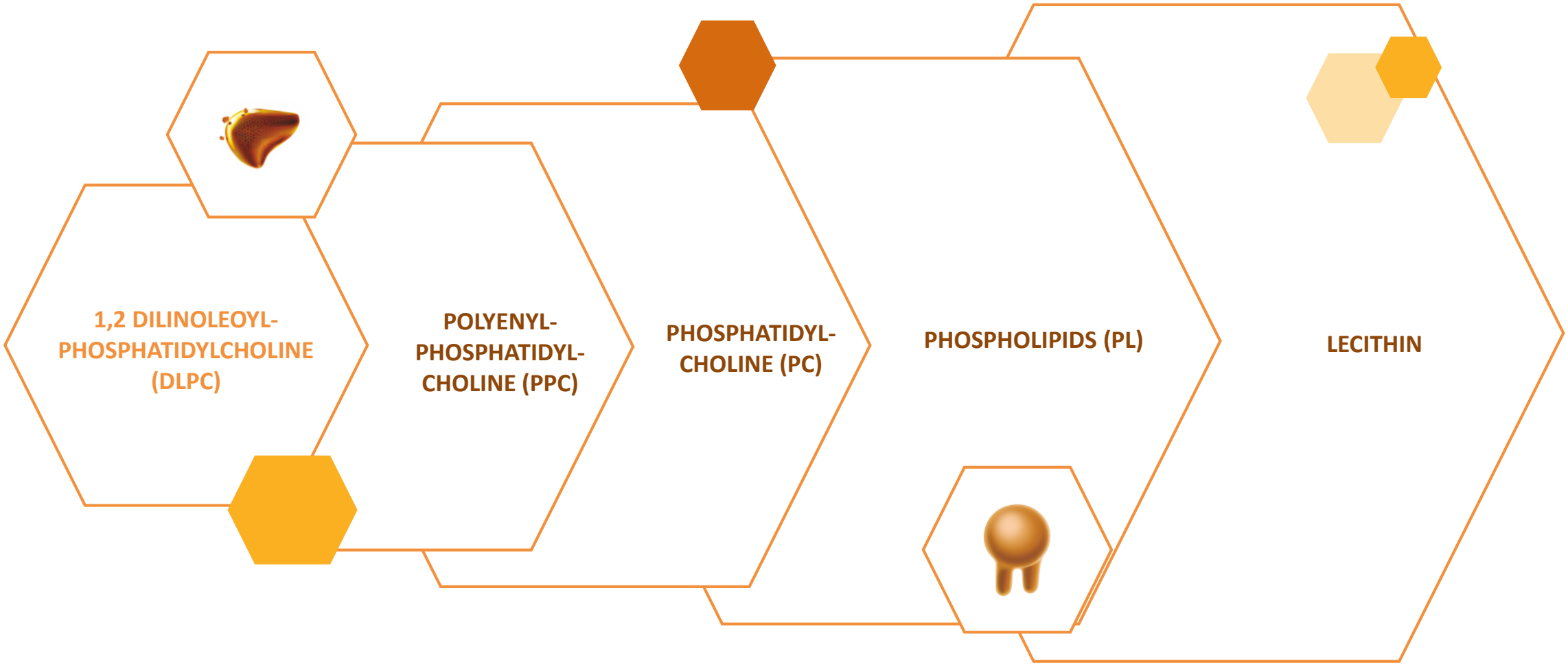

Franciscus A. HCSP. Version 1. April 2015

# General characteristics of essential phospholipids (EPL)

- **PPC** – highly purified extract of polyenylphosphatidylcholine molecues from soybean
- **DLPC** – 1,2-dilinoleoylphosphatidylcholine – main active ingredient
  - The acyl moieties of polyunsaturated phospholipids consist of **essential fatty acids** – hence "essential" phospholipids
  - Characterized as evidence-based medicine by available studies but still require further clinical trials to be conducted
  - Related, relevant side-effects not observed
- **EPL – indications**
  - Liver diseases of various origins:
    - Fatty liver of different origins (non-alcoholic and alcoholic liver disease)
    - Drug hepatotoxicity
    - Adjuvant therapy in chronic viral hepatitis

Gundermann KJ, et al. Pharmacol Rep 2011;63(3):643–59

# DLPC makes EPL different from phospholipids obtained from a normal diet




Gundermann KJ, et al. Pharmacol Rep 2016;3:643–59

# Comparative study on composition of essential phospholipids (PPC)

- 5 commercially available PPC preparations (Essentiale® Forte, Fortifikat, Hepatoprotect Regenerator, Fortifikat Forte, and Esentin Forte)
- Lipid extraction and a comparative analysis of key lipid content (the glycerophospholipid composition) of each PPC

**Results**

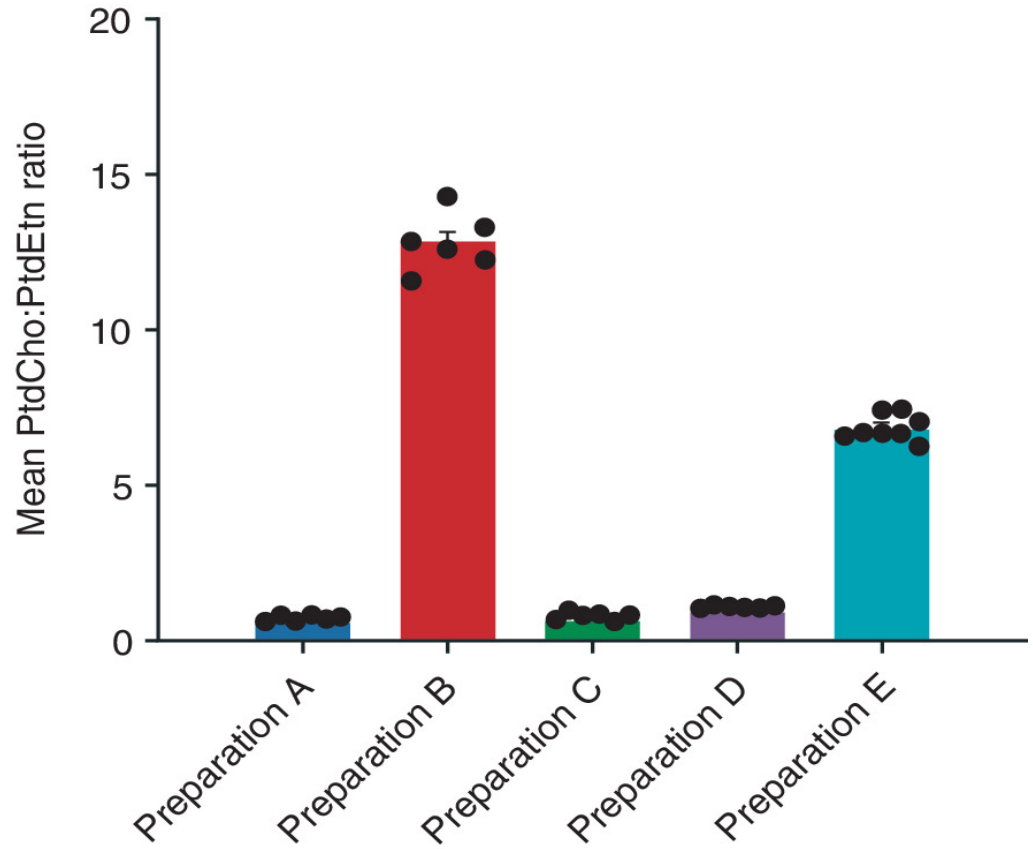
- Lipid composition consistent between the preparations
- The highest phosphatidylcholine (PtdCho) levels (61.9 mol%) and lowest phosphatidylethanolamine (PtdEtn) levels (4.9 mol%) were found in Essentiale® Forte

**Conclusion**

- Essentiale® Forte, compared with the other PPC analyzed, could be considered the most beneficial, commercially-available hepatoprotective product in the treatment of NAFLD

NAFLD, non-alcoholic fatty liver disease; PPC, polyenylphosphatidylcholine
Lüchtenborg C, et al. Lipids 2020;55(3):271–8

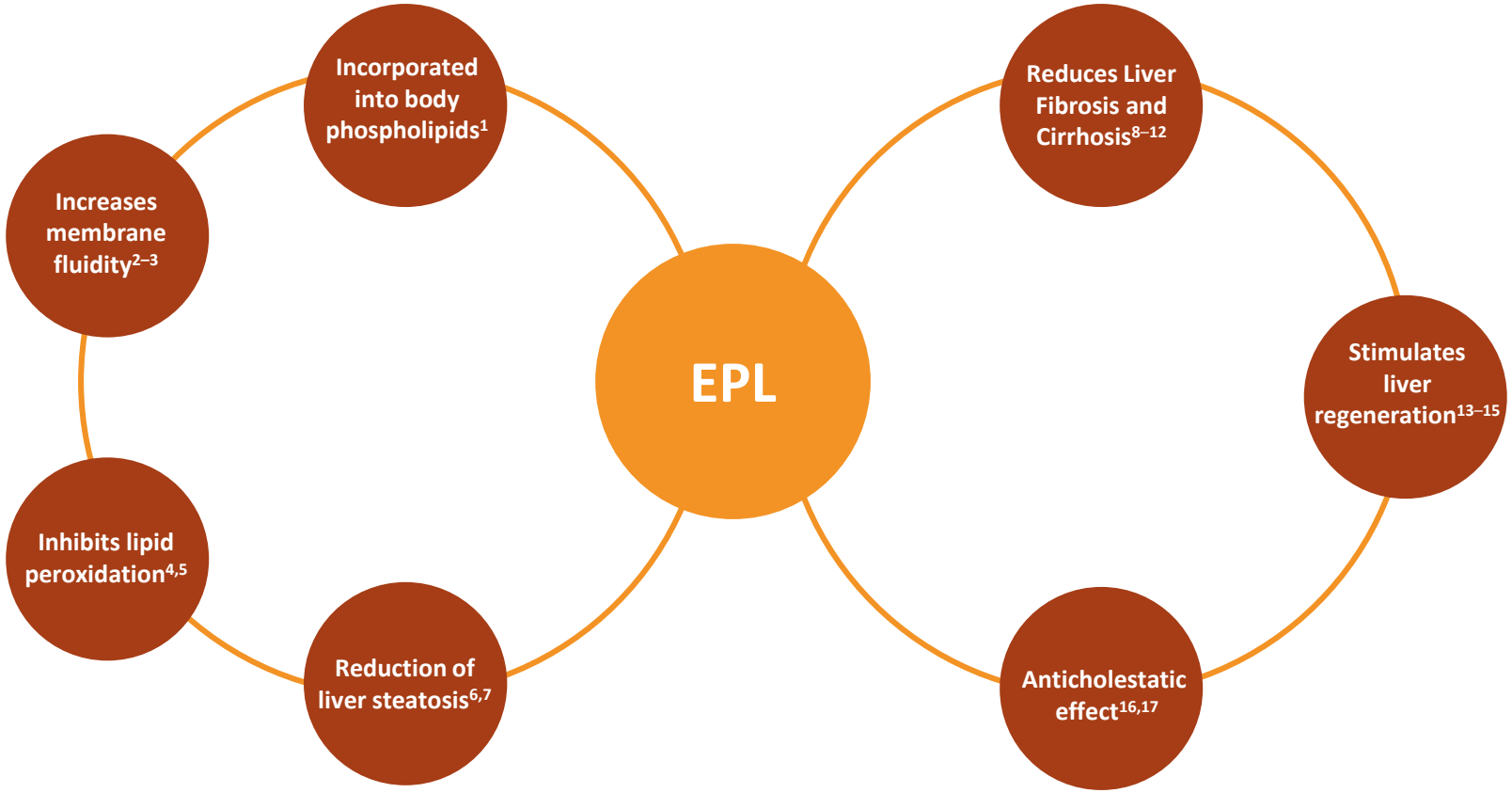
# Comparative study on composition of essential phospholipids (PPC)




A: Esentin Forte
B: Essentiale® Forte 300 mg
C: Fortifikat 500 mg
D: Fortifikat Forte 750 mg
E: Hepatoprotect Regenerator 712.5 mg

PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine
Lüchtenborg C, et al. Lipids 2020;55(3):271–8

# Pre-clinical evidence for EPL




EPL, essential phospholipids

1. Kidd P. Altern Med Rev 1996;1:258–74; 2. Perona JS. Biochimica et Biophysica Acta 2017;1859:1690–703; 3. Zierenberg O, et al. Atherosclerosis 1981;39:527–42; 4. Martelli A, et al. Med Sci Res 1989;17:995–6; 5. Klinger W, et al. Gastroenterol 1991;29(suppl 2):14–7; 6. Jiang Q, et al. Acad J Guangzhou Med Coll 2008;37; 7. Lee HS, et al. Life Sci 2014;118:7–14; 8. Lieber CS, et al. Gastroenterology 1994;106:152–9; 9. Lieber CS, et al. Hepatology 1990;12:1390–8; 10. Li J, et al. Hepatology 1992;15:373–81; 11. Aleynik SI, et al. J Hepatol 1997;27:554–61; 12. Maros T, et al. Arzneimittelforschung 1973;23:1538–42; 13. Holeček M, et al. Arzneimittelforschung 1992;42:337–9; 14. Kropáčová K, et al. Physiol Res 1995;44:241–7; 15. Kožurková M, et al. Radiats Biol Radioecol 1999;39:388–93; 16 Lamireau T, et al. Pediatr Res 2007,61:185–90; 17. Golochevskaia VS, et al. Klin Med (Mosk) 1997;75:30–33

# Modification of membrane lipid composition with EPL protects against the development of insulin resistance?

1. True
2. False

EPL, essential phospholipids

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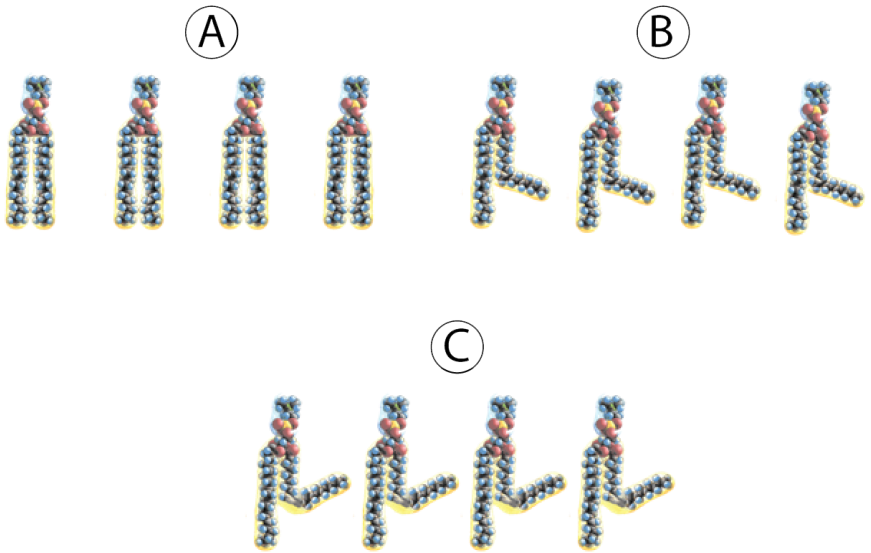
# Mechanisms of action of EPL

## 1. EPL increase membrane fluidity (1/2)

- Changes in the phospholipid FA composition of membranes ➡ changes in the collective physicochemical properties of the bilayer (e.g. flexibility and fluidity) ➡ modulation of the function of membrane proteins mediating insulin action
- **Modification of membrane lipid composition:**
  - Protects against the development of **insulin resistance**
  - Improves some of the altered markers of **metabolic syndrome**




**Saturated fatty acids result in rigid, unresponsive membranes** (A, stearic acid; 18:0)

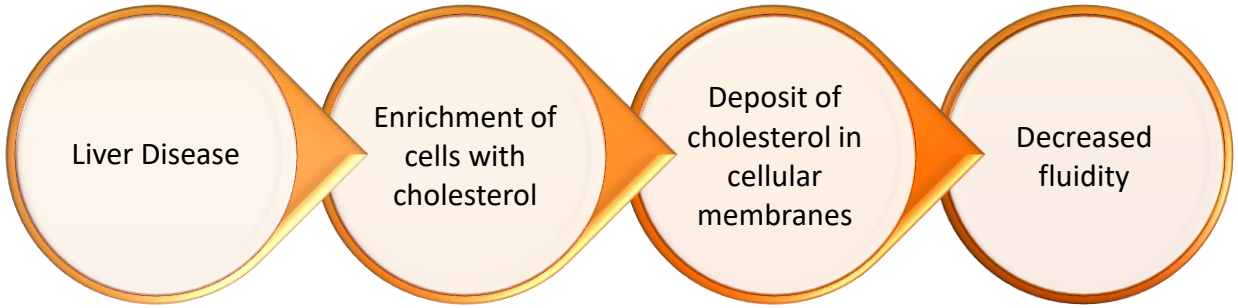
**MUFA** (B, oleic acid) **and PUFA** (C, linoleic acid) **occupy more space leading to more fluid membranes**

EPL, essential phospholipid; FA, fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
Perona JS. Biochimica et Biophysica Acta 2017;1859:1690–703

# Mechanism of action of EPL

## 1. EPL increases membrane fluidity (2/2)

- A study in ageing rats demonstrated that long-term **administration of EPL** (100–300 mg/kg/day orally for 10 weeks) **increased the microsomal polyunsaturated fatty acid content** up to 7 days after treatment cessation[1]
- *In-vitro*, the incorporation of PPC from soya beans to human HDL leads to higher **fluidity of HDL-PPC particles than that of native HDL,** but lower than the fluidity of PPC[1]
- **PPC contributes to increasing membrane fluidity by sequestering excess cholesterol** in rythrocyte membranes[2]




EPL, essential phospholipid; HDL, high-density lipoprotein; PPC, polyenylphosphatidylcholine

1. Dargel R, et al. Gastroenterol J 1991;51:73–7; 2. Zierenberg O, et al. Atherosclerosis 1981;39:527–42; 3. Owen JS, et al. Prog Hepato-Pharmacol 1995;1:168–76

# Mechanism of action of EPL

## 2. EPL inhibit lipid peroxidation

- **Malondialdehyde (MDA) and 4-hydroxinonenal (4-HNE)**
  - **PPC** (600 mg/kg/day orally) **reduced in-vitro MDA** by cultured hepatocytes[1]
  - **DLPC significantly reduced 4-HNE production in rats by 36%** ($P<0.001$)[2]
- **Hydrogen peroxide ($H_2O_2$) and glutathaione**
  - EPL (100–300 mg/kg) **reduced $H_2O_2$ production** by liver microsomes[3]
  - EPL (100–300 mg/kg) **increased the concentration of glutathaione** that scavenges $H_2O_2$[3,4]
  - EPL (100–300 mg/kg) **reduced lipid peroxidation**[3]

EPL, essential phospholipids

1. Martelli A, et al. Med Sci Res 1989;17:995–6; 2. Takeshige U, et al. Hepatology 1996;24:240; 3. Klinger W, et al. Z Gastroenterol 1991;29(Suppl 2):14–17; 4. Gusdon A, et al. Oxidative Medicine and Cellular Longevity 2014;2014:637027

# Mechanism of action of EPL

## 3. EPL prevent liver steatosis

- **EPL increase leptin secretion and reduces lipid accumulation in the liver[1]**
  - Recent studies in rats fed a high-fat diet indicated that treatment with Essentiale® forte (550 mg/kg/day orally) for 4 weeks **reduced lipid content of the liver tissue**
  - This effect was associated with **increased expression of the leptin gene** as shown by **increased levels of leptin mRNA**
- **EPL alleviate HFD-induced hyperlipidemia[2]**

EPL, essential phospholipids; HFD, high-fat diet; NAFLD, non-alcoholic fatty liver disease

1. Jiang Q, et al. Acad J Guangzhou Med Coll 2008;37(10); 2. Lee HS, et al. Life Sci 2014;118:7–14

# Mechanism of action of EPL

## 4. EPL reduce liver fibrosis and cirrhosis

- The **mechanism of the anti-fibrotic** action of EPL may involve:[1–3]
  - **Membrane stabilization**[2]
  - **Stabilization of hepatic stellate cells,** attenuating their transformation to transitional cells[1]
  - **Activation of collagenase enzyme** leading to a reduction in collagen accumulation[1,3,4]
- Studies in animals (rats and baboons) showed that EPL:
  - **Markedly attenuates fibrosis and cirrhosis**[1,4]
  - Decreased hepatic collagen content[5]

EPL, essential phospholipd

1. Lieber CS, et al. Gastroenterology 1994;106:152–9; 2. Lieber CS, et al. Hepatology 1990;12:1390–8; 3. Li J, et al. Hepatology 1992;15:373–81;
4. Aleynik SI, et al. J Hepatol 1997;27:554–61; 5. Maros T, et al. Arzneimittelforschung 1973;23:1538–42

# Mechanism of action of EPL

## 5. EPL stimulate liver regeneration

- In rats subjected to a partial hepatectomy or sublethal irradiation in several studies, EPL:
  - **Stimulated liver regeneration** as manifested by higher mitotic activity and lower triglyceride levels[1]
  - **Protected the genetic material** as shown by a reduction in the frequency of chromosomal aberrations[2]
  - EPL also increased liver DNA content, owing to a more rapid growth of liver remnant after Essentiale® treatment[3]

**In addition to its stabilizing effect on cell membranes, EPL also exert some protective effects on the genetic material[3]**

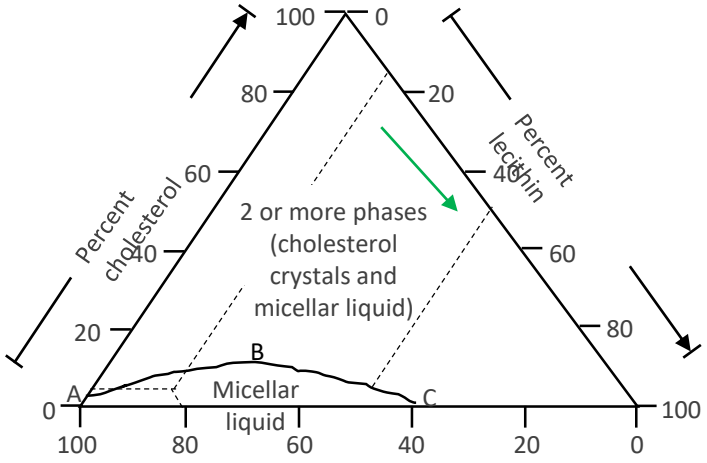
EPL, essential phospholipids

1. Holeček M, et al. Arzneimittelforschung 1992;42:337–9; 2. Kropáčová K, et al. Physiol Res 1995;44:241–7; 3. Kožurková M, et al. Radiats Biol Radioecol 1999;39:388–93

# Mechanism of action of EPL

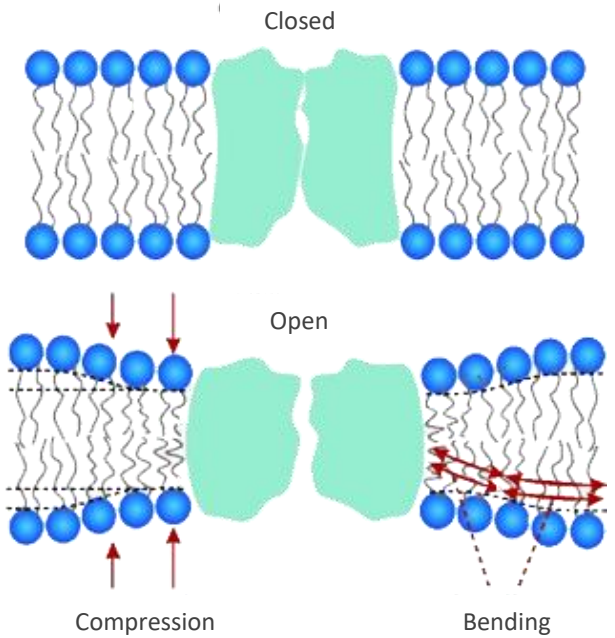
## 6. EPL has an anticholestatic effect, through:

### Bile stabilization



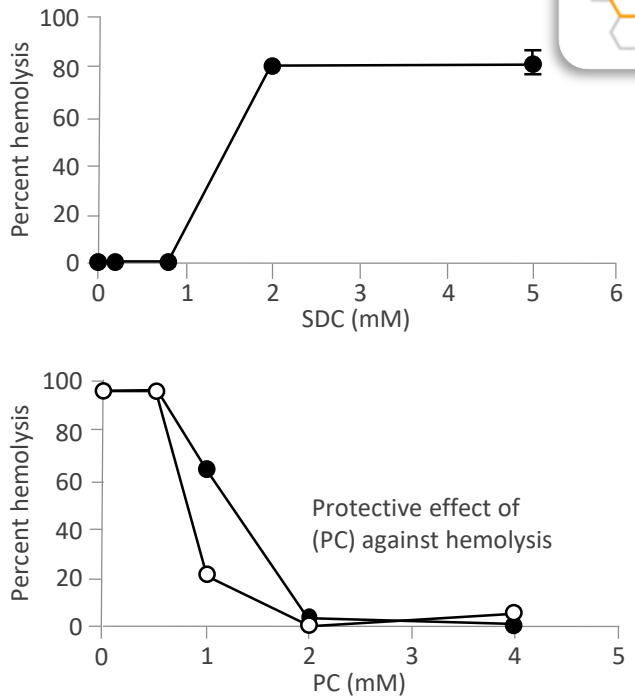

The decrease of PPC concentration leads to bile destabilization[1]

### Secretion




Bile acids are secreted by adenosine triphosphate-binding cassettes (ABC). The activity of cassettes depend on membrane fluidity. PPC improves membrane fluidity and elasticity[2]

### Protection



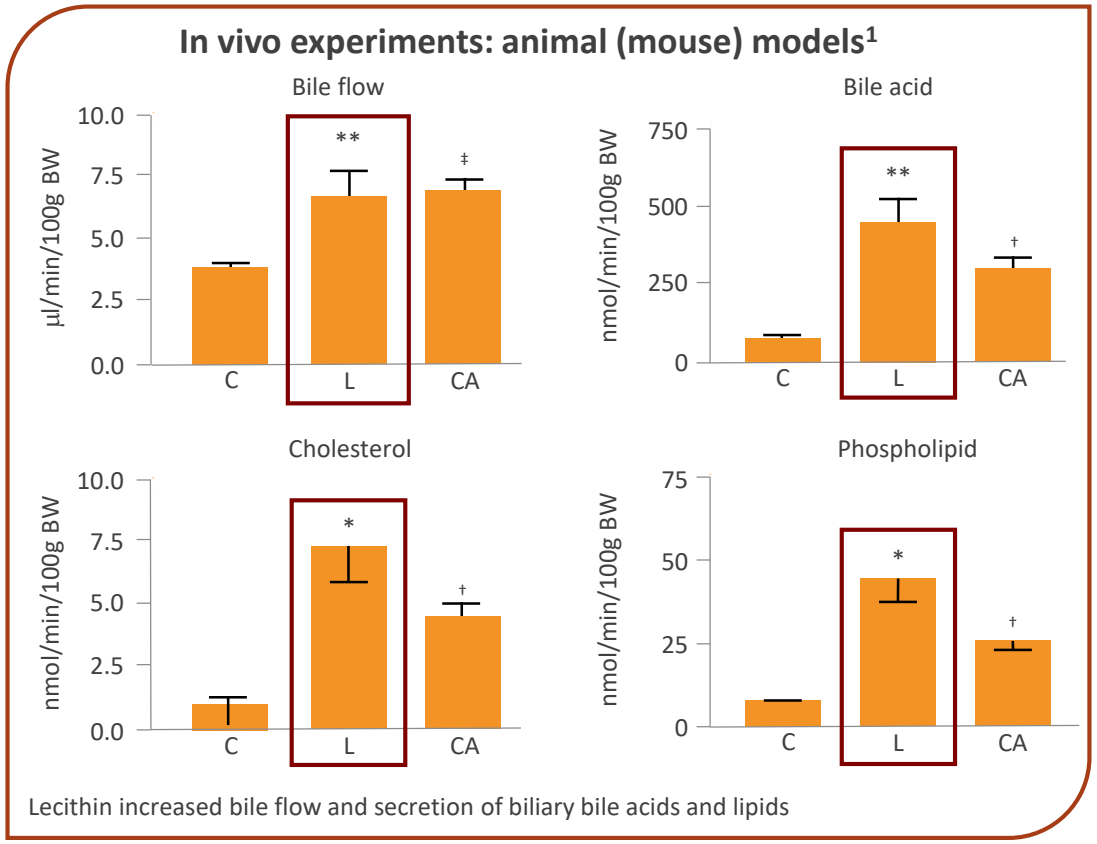

PPC can directly protect cell membranes from bile salt-induced injury (*in vitro*)[2]

EPL, essential phospholipids; PPC, polyenylphosphatidylcholine

1. Lundbæk JA, et al. J R Soc Interface 2010;7(44):373–95; 3. Dial EJ, et al. J Gastroenterol Hepatol 2008;23(3):430–6

# Mechanism of action of EPL

## 6. The anticholestatic effect of EPL was proven in *in-vivo* experiments[1] and clinical trials[2]



Lecithin increased bile flow and secretion of biliary bile acids and lipids

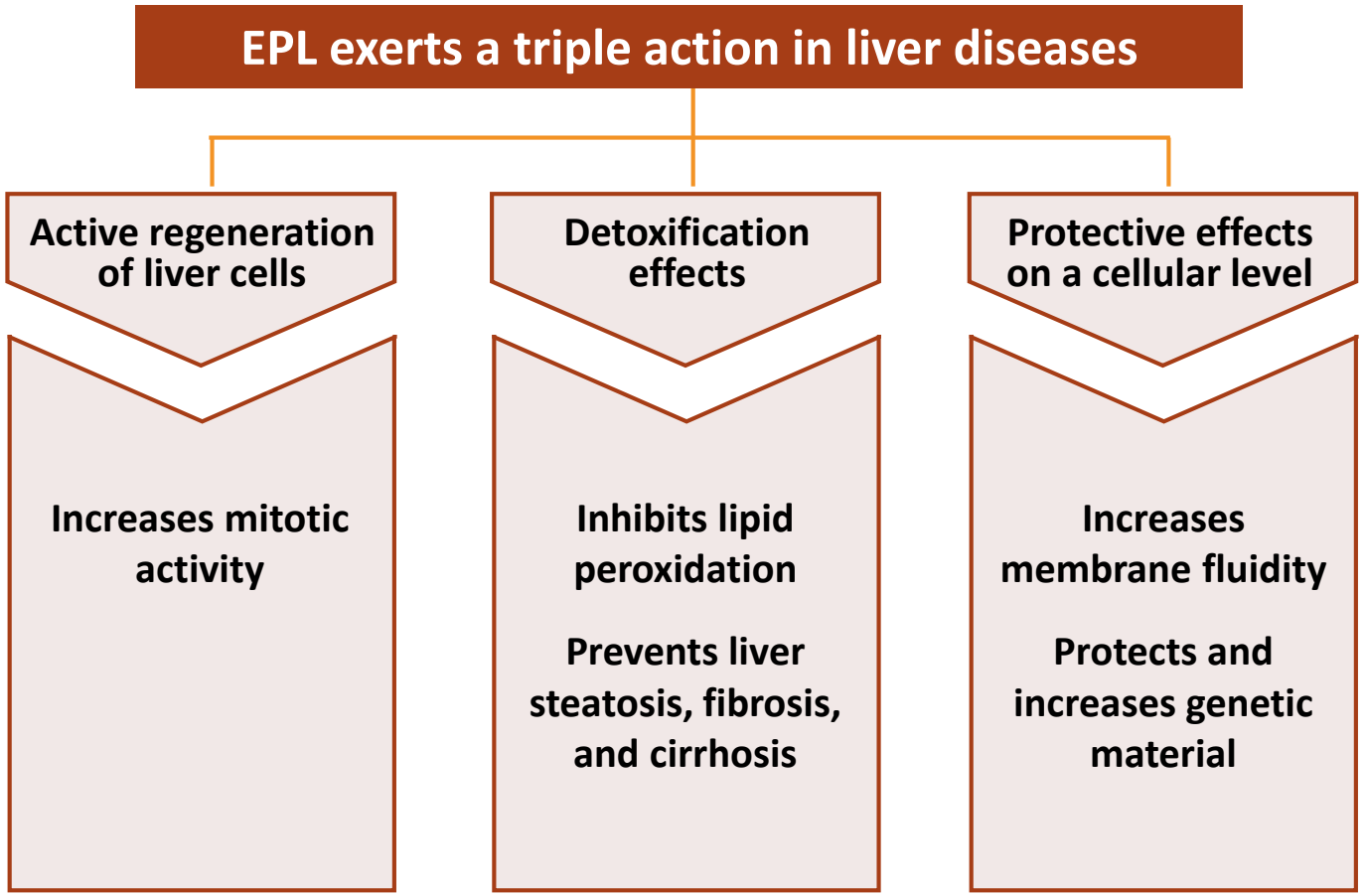
### Clinical trials

- Phospholipids given to patients presenting with dramatic circulatory problems and simultaneous hepatic disorders, led to **positive changes in intrahepatic cholestasis and liver function[3]**

*p<0.05 when compared with C diet; **p<0.01 when compared with C diet; †p<0.01 when compared with C diet. C, control; L, lecithin, CA, cholic acid; EPL, essential phospholipids

1. Lamireau T, et al. Pediatr Res 2007,61(2):185–90; 2. Daniela Küllenberg, et al. Lipids Health Dis 2012;11:3; 3. Golochevskaia VS, et al. Klin Med (Mosk) 1997;75(5):30–3

# Summary of the mechanism of action of EPL

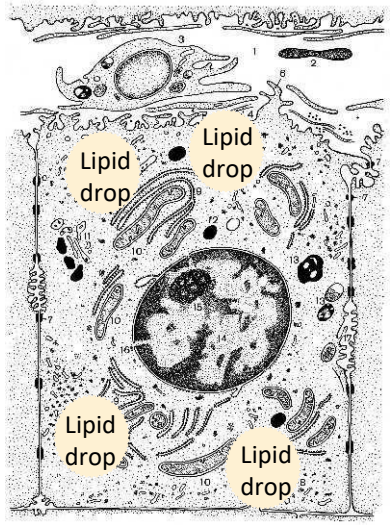



1. Phosphatidylcholine_CCSI; 2. Demirbilek S, et al. Hepatol Res 2006;34:84–91; 3. Martelli A, et al. Med Sci Res 1989;17:995–6

# Effect of PPC on biochemical pathways in hepatocytes (1/3)

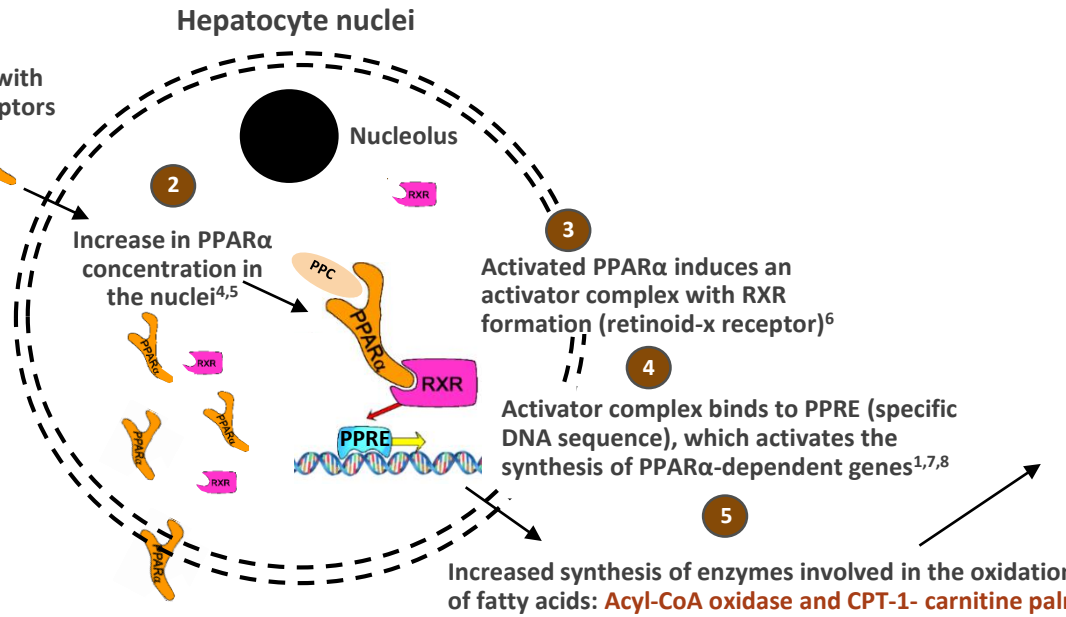
**Hepatocyte with steatosis**

Lipid accumulation in hepatocyte cytoplasm



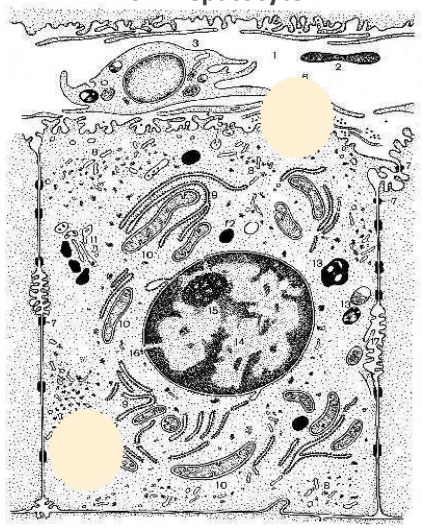

Detailed view of processes occurring in the nuclei

**Up-regulation of beta-oxidation of fatty acids in liver cells**




6

**Gradual elimination of lipids from hepatocyte[9]**



**Increased synthesis of enzymes involved in the oxidation of fatty acids in the liver**

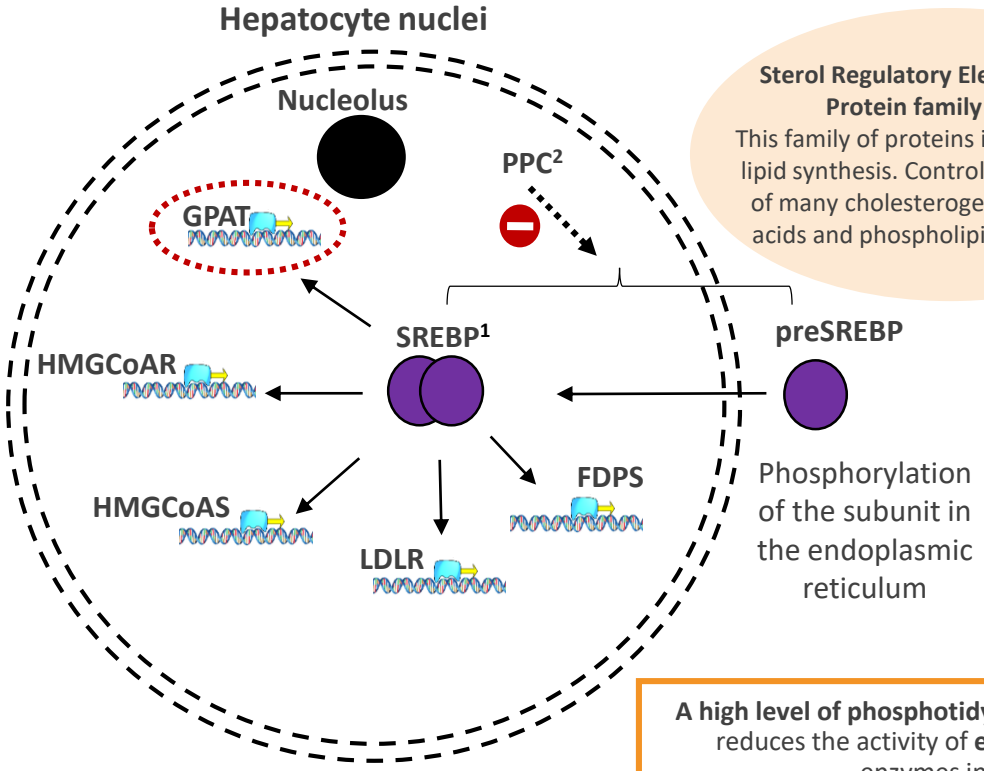
- **Acyl-CoA oxidase[1]** → Participates in the oxidation of fatty acids
- **Carnitine palmitoyltransferase[9,10]** → Responsible for the transfer of fatty acids from the outer membrane to the inner membrane for oxidation of fatty acids inside the mitochondria

PPC, polyenylphosphatidylcholine

1. Manu V, et al. Cell 2009;138:476–88; 2. Mehedint MG, et al. Curr Opin Clin Nutr Metab Care 2013;16:339–45; 3. Mello T, et al. PPAR Research 2016;2016:7403230; 4. Mikael LG, et al. J Nutr 2012;142:411–8;
6. Prufer K, et al. Mol Endocrinol 2002;16:1738–51; 7. Maruvada, P, et al. J Biol Chem 2003;278:12425–32; 8. Chandra V, et al. Nature 2008;456:350–6; 9.Tzeng J, et al. PLoS ONE 2015;10:e0134996; 10. Lamaziere A, et al. Gastroentrologie Clinique et Biologique 2010;34:250—1; 11. Buang Y, et al. Nutrition 2005;21:867–73

# Effect of PPC on biochemical pathways in hepatocytes (2/3)

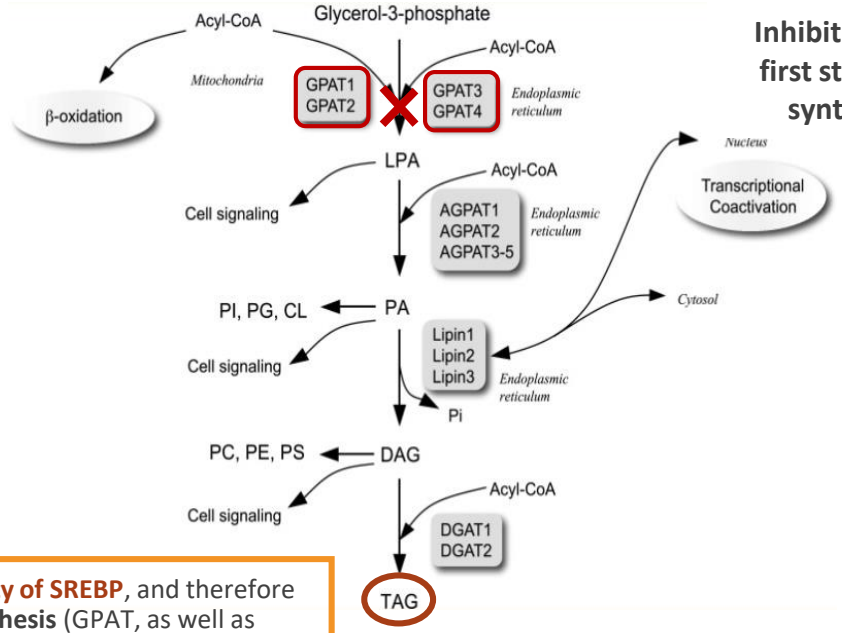
Detailed view of processes occurring in the nuclei




**Sterol Regulatory Element-Binding Protein family (SREBP)**
This family of proteins is responsible for lipid synthesis. Controls the expression of many cholesterogenic genes, fatty acids and phospholipid biosynthesis[2]

## Inhibition of triglyceride synthesis in liver cells

**It is believed that excessive activity of SREBP can lead to increased synthesis of TG, which contributes to the development of insulin resistance, metabolic syndrome and fatty liver disease[2]**




**Inhibition of the first stage of TG synthesis[3,4]**

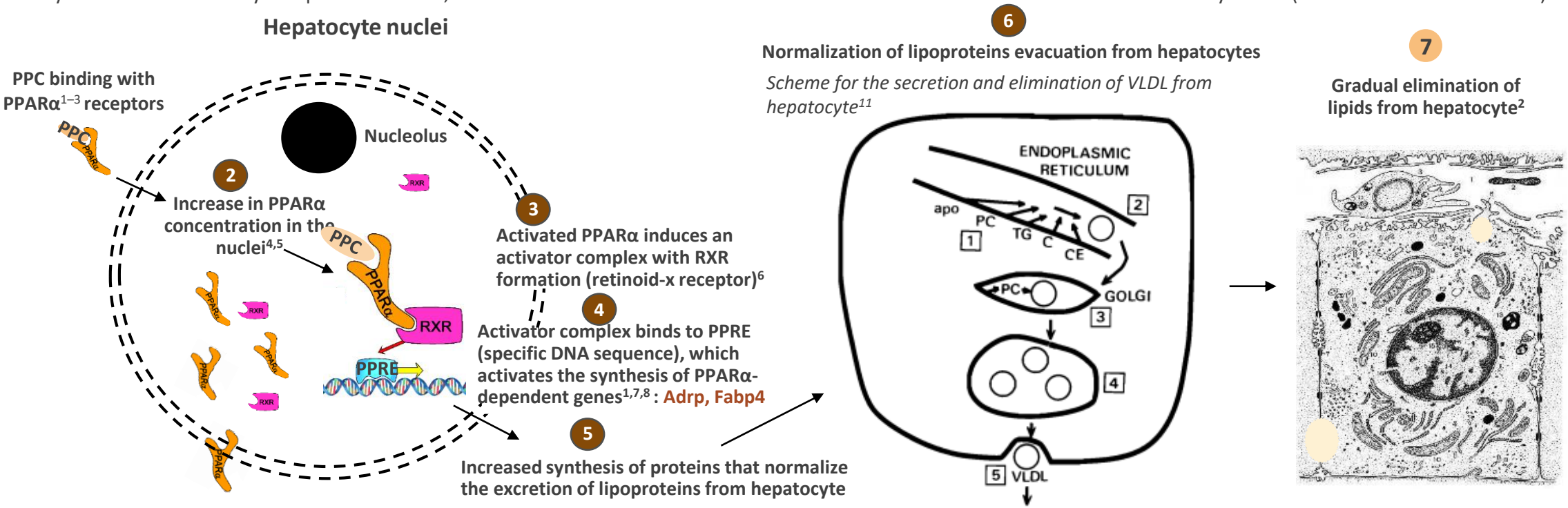
**A high level of phosphotidylcholine reduces the activity of SREBP**, and therefore reduces the activity of **enzymes involved in TG synthesis** (GPAT, as well as enzymes involved in the synthesis of cholesterol)

SREBP, transcriptional factor sterol regulatory element binding protein; preSREBP, precursor factor of SREBP; HMGCoAS, HMG-CoA synthetase; HMGCoAR , HMG-CoA reductase; FDFS, farnesyl diphosphate synthase; SS, squalene synthetase; LDLR, LDL particle receptor

1. Н.А. Колчанов и др.Генные сети липидного метаболизма. БЮЛЛЕТЕНЬ СО РАМН, №2 (120), 2006 г; 2. Walker AK, et al. Cell 2011;147:840–52; 3. Matías Caviglia J, et al. J Lipid Res 2004;45:15–9; 4. Takeuchi K, et al. Am J Physiol Endocrinol Metab 2009;296:E1195–E1209

# Effect of PPC on biochemical pathways in hepatocytes (3/3)

The mechanisms responsible for the development of fatty liver disease with choline deficiency are associated with impaired formation of VLDL and their excretion from the liver. Phosphatidylcholine is a necessary component of VLDL, VLDL can't be excreted from the liver without PPC and therefore accumulates in the cytosol[14] (PPC content in VLDL is 10%)[10]




**ADRP** – hepatocyte differentiation protein: proven effect on VLDL secretion[11,12]

**FABP4** – family of transporters of fatty acids and other lipophilic substances. These proteins promote the transfer of fatty acids through extra- and intracellular membranes[12]

PPC, polyenylphosphatidycholine; VLDL, very low-density lipoprotein

1. Chakravarthy MV, et al. Cell 2009;138:476–88; 2 Mehedint MG, et al. Curr Opin Clin Nutr Metab Care 2013;16:339–45; 3. Mello T, et al. PPAR Research 2016;2016:7403230; 4. Prufer K, et al. Mol Endocrinol 2002;16:1738–51; 5. Maruvada, P, et al. J Biol Chem 2003;278:12425–32; 6. Chandra V, et al. Nature 2008;456:350–6; 7. Tzeng J, et al. PLoS ONE 2015;10:e0134996; 8. Lamaziere A, et al. Gastroentrologie Clinique et Biologique 2010;34:250—1; 9. Walker HK, et al. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths, 1990. Chapter 31; 10. Vance JE, et al. Canadian J Biochem Cell Biology 1985;63:870–81; 11. Choi SH, et al. Trends Endocrinol Metab 2011;22:353–63; 12. Csak T, et al. PLoS One 2015;10:e0129251

# Summary of the suggested mechanism of action of EPL in hepatic steatosis reduction

**PPC potential impact on TG metabolism and transport**

## Increased TG oxidation

**PPARα**[1,2]

- **Acyl-CoA oxidase**[1]
- **CPT-1- carnitine palmitoyltransferase**[1,3,4]

## Decreased TG synthesis

- **ME- Malate dehydrogenase**[3]
- **G6PDH- Glucose-6-phosphate dehydrogenase**[3]
- **FAS-fatty acid synthase**[3]

**PPC/choline regulate epigenetic mechanisms modify liver function**

**PPARγ**[1,5] *Contradictive data*

**or**

**Sterol Regulatory Element-Binding Proteins (SREBPs)**[6]

This protein can activate genes involved in the synthesis and trafficking of cholesterol and other lipids (activate enzymes) ↓ But PPC can decreased this protein activity

**GPAT- Glycerol-3-phosphate O-acyltransferase**[7]

- **ACS-acyl-CoA synthetase**[7]

## Increased lipoprotein excretion

- Binding with **PPARα** regulates the expression of genes responsible for the storage and secretion of LDL/VLDL: ***Adrp, Dgat2, Cpt1a and Fabp4***[8–10]
- Normalized HDL level[10–13]

PPC, polyenylphosphatidylcholine; TG, triglyceride

1. Chakravarthy MV. Cell 2009;138:476–88; 2. Lamaziere A, et al. Gastroentйrologie Clinique et Biologique 2010;34:25–1; 3. Buang Y, et al. Nutrition;2005;21:867–73; 4. Mello T, et al. PPAR Res 2016;2016:7403230; 5. Mihai G, et al. Curr Opin Clin Nutr Metab Care 2013;16:339–45; 6. Walker AK, et al. Cell 2011;147:840–52; 7. Matías Caviglia J, et al. J Lipid Res 2004;45:1500–9; 8. Csak T, et al. PLoS One 2015;10:e0129251; 9. Yao ZM, et al. J Biol Chem 1988;263:2998–3004; 10. Jacobs RL, et al. J Biol Chem 2008;283:2147–55; 11. Galli C, et al. Lipids 1985;20:561–6; 12. Navder KP, et al. Life Sci 1997;61:1907–14; 13. Zierenberg O, et al. Atherosclerosis 1981;39:527–42

# An overview of essential phospholipids in liver diseases

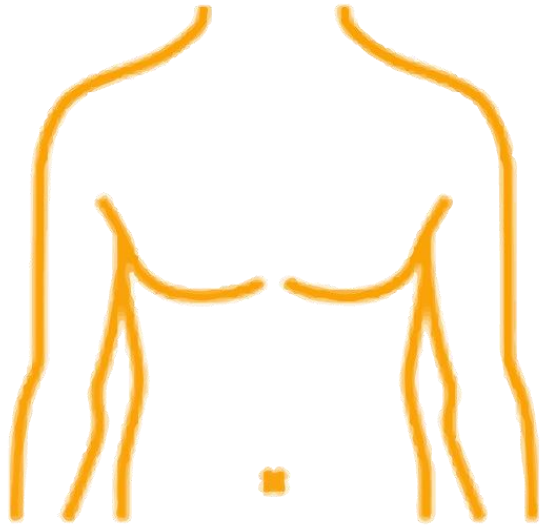
| EPL have multiple modes of action in liver diseases | |
|---|---|
| Restore cell membrane structure | Stimulate liver regeneration |
| Increase cell membrane fluidity | Correct or inhibit fibrogenic processes |
| Enhance membrane-associated metabolic functions | Influence apoptosis |
| Reduce or normalize peroxidative reactions | Stabilise bile composition |
| Decrease cytolysis | Modulate lipid metabolism |
| Improve excretory, detoxifying/clearing, and synthesizing capacity of the liver | Diminish or abolish fatty infiltration and hepatocyte necrosis |
| Improve immune properties | Decrease experimental hepatocarcinogenesis |

EPL, essential phospholipids
Gundermann KJ. Pharmacol Rep 2011;63:643–59

SANOFI

# Essential phospholipids in treating NAFLD/NASH

- Available evidence from clinical studies demonstrate the efficacy of EPL in the management of NAFLD/NASH with or without T2DM
  - Improves liver structure by decreasing the progression of fatty infiltration of the liver and slowing down hepatic fibrogenesis and steatosis[1,2]
  - Reduces liver size[3]
  - Improves liver function through a reduction in transaminase levels[3]
  - Reduces triglycerides and cholesterol levels[4]
  - Improves clinical status of patients[3]



**EPL have a complex effect on the whole body[3]**

EPL, essential phospholipids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, Type 2 diabetes mellitus

1. Gundermann KJ, et al. Pharmacol Rep 2011;63:643–59; 2. Lee HS, et al. Life Sciences 2014;118:7–14; 3. Gundermann KJ, et al. Clin Exp Gastroenterol 2016;9:10 17; 4. Holecek M, et al. Arzneimittelforschung 1992;42:337–9

# Available evidence for essential phospholipids in ALD

## Beneficial effect of oral Essentiale® forte in 5 studies

| Study | Comparator | ALT | AST | Response rate | Histology | Ultra-sonography | Symptoms |
|---|---|---|---|---|---|---|---|
| **Knüchel 1979** | Placebo | ✓ | ✓ | ND | ✓ | ND | ND |
| **Xu 2007** | Inosine + Vitamin C | ✓ | ✓ | ND | ✓ | ✓ | ✓ |
| **Sas 2011** | Vitamin E | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Schüller-Pérez 1985** | Placebo | ✓ | ✓ | ✓ | ND | ND | ND |
| **Hazuka 1987** | Vitamin therapy | ✓ | ✓ | ND | ND | ND | ND |

✓ = Statistically significant difference observed in favour of Essentiale® forte; ND=endpoint not documented in the study
ALD, alcoholic liver disease

# Which mechanism of action would best describe the current understanding of the pre-clinical effects of EPL in NAFLD?

1. EPL inhibit lipid peroxidation
2. EPL reduces hepatic fibrosis and cirrhosis
3. EPL reduce hepatic steatosis
4. EPL modulate lipid metabolism
5. EPL increase cell membrane fluidity
6. 3 + 5
7. All of the above

EPL, essential phospholipids; NAFLD, non-alcoholic fatty liver disease

# Conclusions

**Essential phospholipids demonstrate a:**

1. Favorable safety profile[1]
2. Low risk of adverse events[2]
3. Low risk of drug interactions[2]
4. Valuable adjuvant therapeutic option for fatty liver disease (ALD, NAFLD)[3]

ALD, alcoholic fatty liver disease; NAFLD non-alcoholic fatty liver disease

1. Gundermann KJ, et al. Clin Exp Gastroenterol 2016;9:10 17; 2. Gundermann KJ, et al. Pharmacol Rep 2011;63:643–59; 3. Lüchtenborg C, et al. Lipids 2020;55:271–8


# Q&A


# Break

*Meeting will resume in 30 minutes*