3rd GLOBAL LIVER HEALTH FORUM

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The potential molecular mechanism of EPL; insights from EASL 2022

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Disclosures

- Gert Fricker is an employee of Ruprecht-Karls University of Heidelberg and has no conflicts of interest

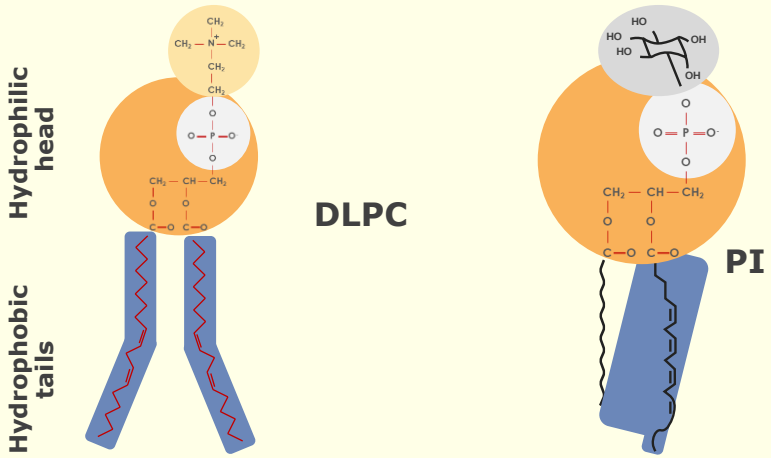
Agenda

1. Introduction to EPL
2. *In vitro* effect of EPL on pro-inflammatory cytokines
3. *In vitro* effect of EPL on lipid metabolising enzymes
4. Summary

Introduction to EPL

Administration of EPLs have demonstrated changes to phospholipid content in cell membranes

- The most abundant phospholipids in mammalian tissues are **phosphatidylcholine (PC)** and **phosphatidylethanolamine (PE)**:[1]



- DLPC, a PPC, is an abundant molecule found in some EPLs[2]
- PI is also a small constituent seen in some EPLs[3]

Respective EPL administration significantly increases the percentage of DLPC in hepatic cell membranes[2]

DLPC, 1,2-di-linoleoyl phosphatidylcholine; EPL, essential phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol

1. Li Z, et al. Cell Metab 2006;3(5):321–31; 2. Gundermann KJ, et al. Clin Exp Gastroenterol 2016;9:105–17; 3. Wupperfeld D, et al. EASL. 2022.

There are limited studies investigating the MoA of EPL in human hepatocytes

Administration of **EPL** rich in PC has demonstrated **lipid-regulating properties**, as well as multiple **membrane protective effects** including **reductions in**:[1–3]

Apoptosis

Oxidative stress

Fibrogenesis

Steatosis

Inflammation

EPL, essential phospholipid; MoA, mechanism of action; PC, phosphatidylcholine

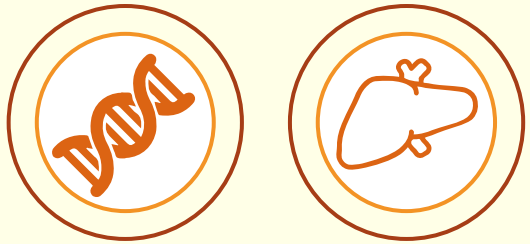
1. Gundermann KJ, et al. Pharmacol Rep. 2011;63(3):643–59; 2. Gundermann KJ, et al. Clin Exp Gastroenterol. 2016;9:105–17; 3. Dajani AI and Abuhammour A. Drugs Ther Perspect. 2021;37:249–64.

In vitro effect of EPL on pro-inflammatory cytokines

Role of HepaRG cell lines in investigating liver functions

HepaRG cell lines originate from the liver of a human donor and have **many potential uses**:[1]

- Can be used to explore metabolism-dependent genetic toxicity and liver toxicity, including assessing toxicity of **specific mechanisms** such as **steatosis**[1]
- Useful to evaluate drugs and perform **drug metabolism studies**, as many detoxifying enzymes are expressed and functional[2]
- Represent a valuable alternative to *ex-vivo* cultivated primary human hepatocytes (PHH), as HepaRG cells share **similar features** with **adult hepatocytes**[2]



Based on gene expression in HepG2, HepaRG and PHH, **HepaRG** cell lines more **closely resemble PHH** and **liver tissues** compared with HepG2 cells[3–5]

PHH, primary human hepatocytes

1. Andersson T, et al. Expert Opin Drug Metab Toxicol 2012;8(7):909–20; 2. Marion MJ, et al. Method Mol Biol 2010;640:261–72; 3. Hart S, et al. Drug Metab Dispos 2010;38(6):988–94; 4. Jennen D, et al. Toxicol Sci 2010;115(1)66–79; 5. Gerets H, et al. Cell Biol Toxicol 2012;28(2):69–87

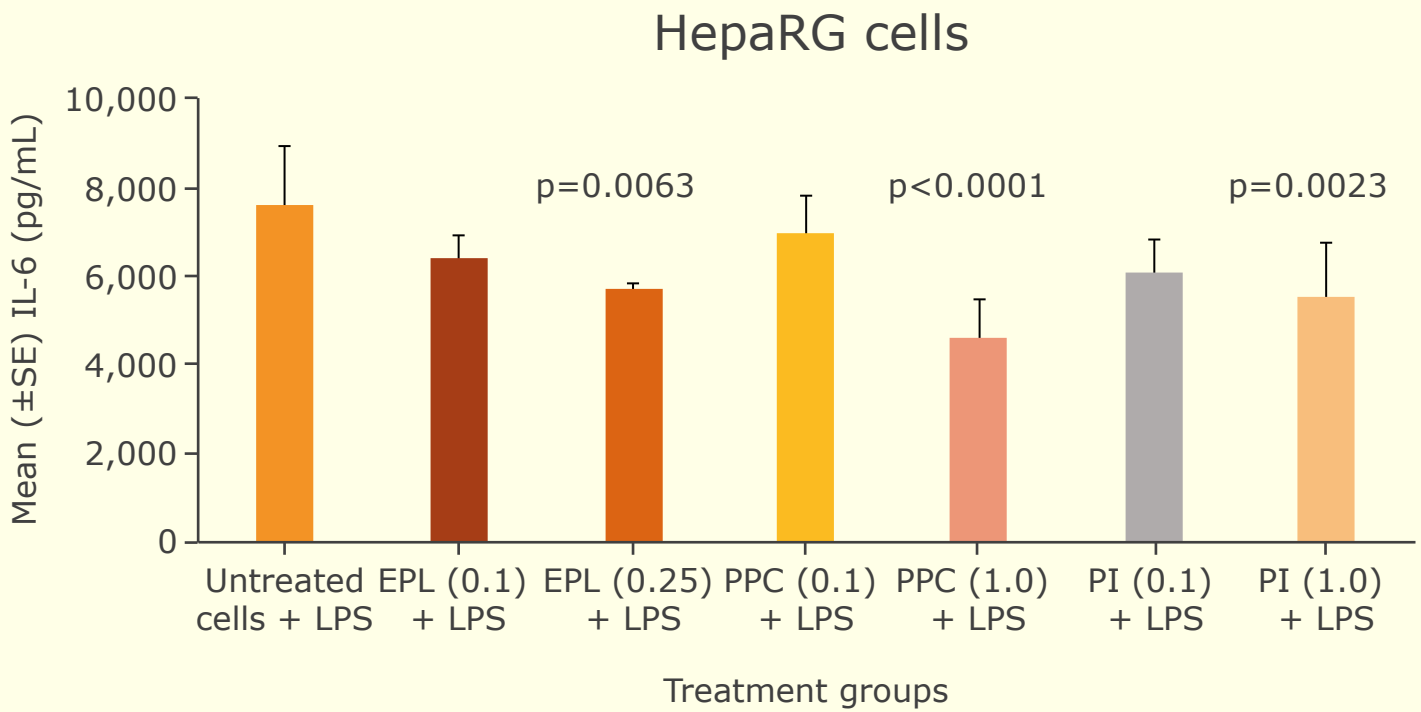
An *in vitro* study was conducted on human cell lines to assess the molecular MoA of EPL

- HepaRG cells were treated with stearic acid and oleic acid to induce steatosis
- Lipopolysaccharide (LPS) and lipid metabolizing enzymes were added to the culture to stimulate the release of pro-inflammatory cytokines
- Effects of non-toxic concentrations of EPL (0.1 and 0.25 mg/mL), PPC and PI (both at 0.1 and 1 mg/mL) on the release of the pro-inflammatory cytokines and G6PD were assessed

EPL, essential phospholipid; G6PD, glucose-6-phosphate dehydrogenase; MoA, mechanism of action; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine

Wupperfeld D, et al. EASL. 2022.

EPL, PPC and PI significantly reduced the release of IL-6 in LPS-treated HepaRG cells



Reduction in the pro-inflammatory cytokine IL-6 supports the anti-inflammatory properties of EPL and two of its components (PPC and PI)

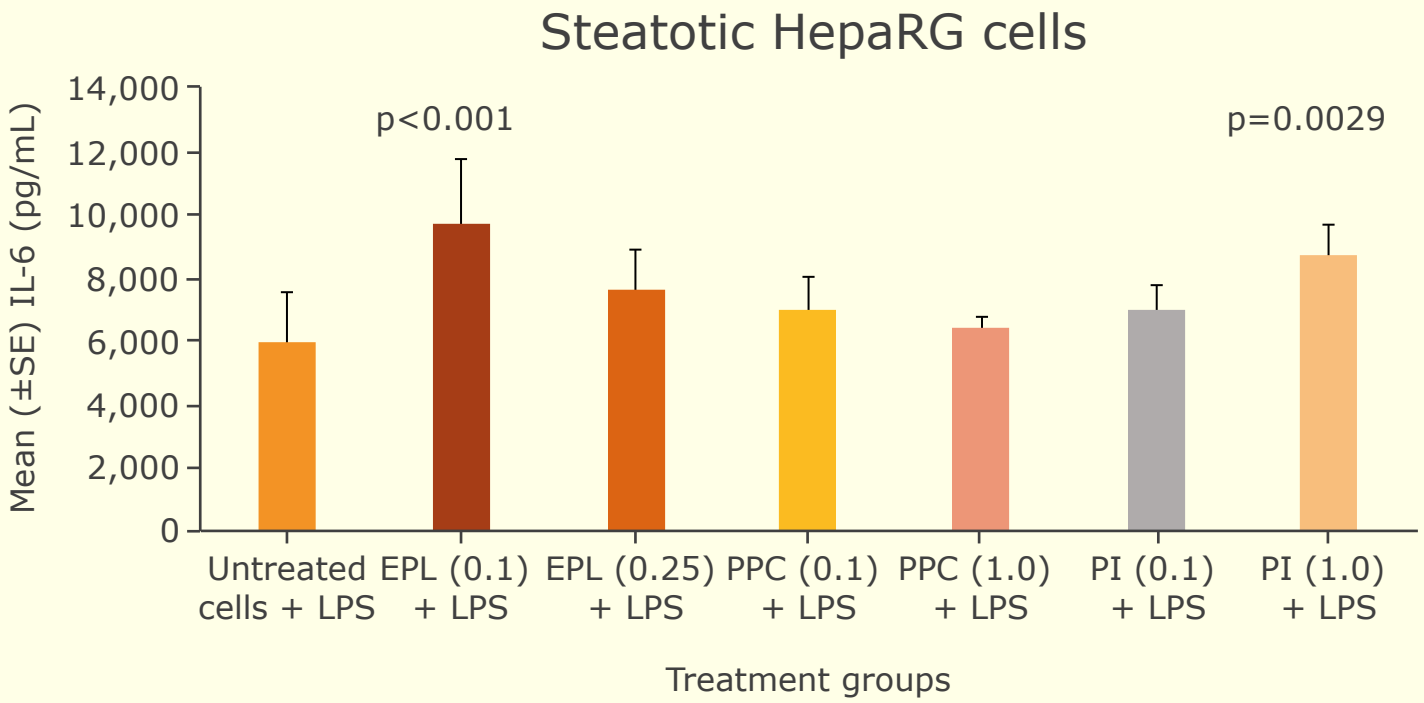
All drug doses presented in mg/mL
EPL, essential phospholipid; IL-6, interleukin-6; LPS, lipopolysaccharide; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine; SE, standard error
Wupperfeld D, et al. EASL. 2022.

Do you think phospholipid treatment increased or decreased IL-6 secretion in steatotic HepaRG cells?

1. Increased
2. Decreased
3. Unsure

IL-6, interleukin-6

EPL and PI increased the release of IL-6 in steatotic, LPS-treated HepaRG cells



This trend was also seen for IL-8 secretion, which was increased in both LPS-treated HepaRG and LPS-treated steatotic HepaRG cells with EPL, PPC and PI treatment ($p<0.001$)

IL-6 activity in steatotic cells may have a protective effect against the further development of steatosis

All drug doses presented in mg/mL
EPL, essential phospholipid; IL-6, interleukin-6; IL-8, interleukin-8; LPS, lipopolysaccharide; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine; SE, standard error
Wupperfeld D, et al. EASL. 2022.

In vitro effect of EPL on lipid metabolising enzymes

G6PD activity was unaffected by phospholipid treatment, whereas ACOX1 expression was reduced in HepaRG cells

G6PD:[1,2]

- NADPH is essential for fatty acid biosynthesis
- G6PD is involved in the pentose phosphate pathway that produces NADPH
- Increased G6PD has been linked with fatty liver in rats

ACOX1:[4,5]

- Rate-limiting enzyme in fatty acid oxidation
- In mice, mutated ACOX1 was associated with NAFLD progression
- Patients with NAFLD had higher hepatic ACOX1 expression than those with healthy livers

G6PD activity **remained unchanged** with EPL, PPC and PI treatment in LPS-treated **HepaRG and steatotic HepaRG cells**[3]

ACOX1 expression **remained unchanged** with EPL, PPC and PI treatment in LPS-treated **steatotic HepaRG cells**[3]

ACOX1 expression **decreased** with EPL, PPC and PI treatment in LPS-treated **HepaRG cells** ($p<0.01$)[3]

In rats with induced fatty liver, those receiving dietary PC had similar hepatic G6PD activity to controls without PC or fatty liver[2]

In mouse ALD models, treatment with PPC downregulated ACOX1[4]

ACOX1, acyl Coenzyme A oxidase; ALD, alcoholic liver disease; EPL, essential phospholipid; G6PD, glucose-6-phosphate dehydrogenase; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NADPH, Nicotinamide adenine dinucleotide phosphate; PC, phosphatidylcholine; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine

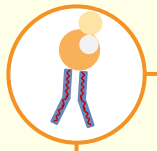
1. Park J, et al. Mol Cell Biol. 2005;25:5146–57; 2. Buang Y, et al. Nutrition. 2005;21:867–73; 3. Wupperfeld D, et al. EASL. 2022; 4. Zeng J, et al. J Biol Chem. 2017;292(9):3800–9; 5. Moreno-Fernandez ME, et al. JCI Insight. 2018;3(6):e93626

FAS activity is associated with saturated fatty acid biosynthesis

The build-up of fat in NAFLD is caused by an excess of triglycerides and fatty acids accumulating in the liver[1]

Fatty acids may influx into the liver following the peripheral lipolysis associated with metabolic comorbidities of NAFLD[1]

Additionally, fatty acids can be synthesised within the liver, further contributing to accumulation of fat deposits and steatosis[1]



FAS is considered a key enzyme in fatty acid biosynthesis[2]

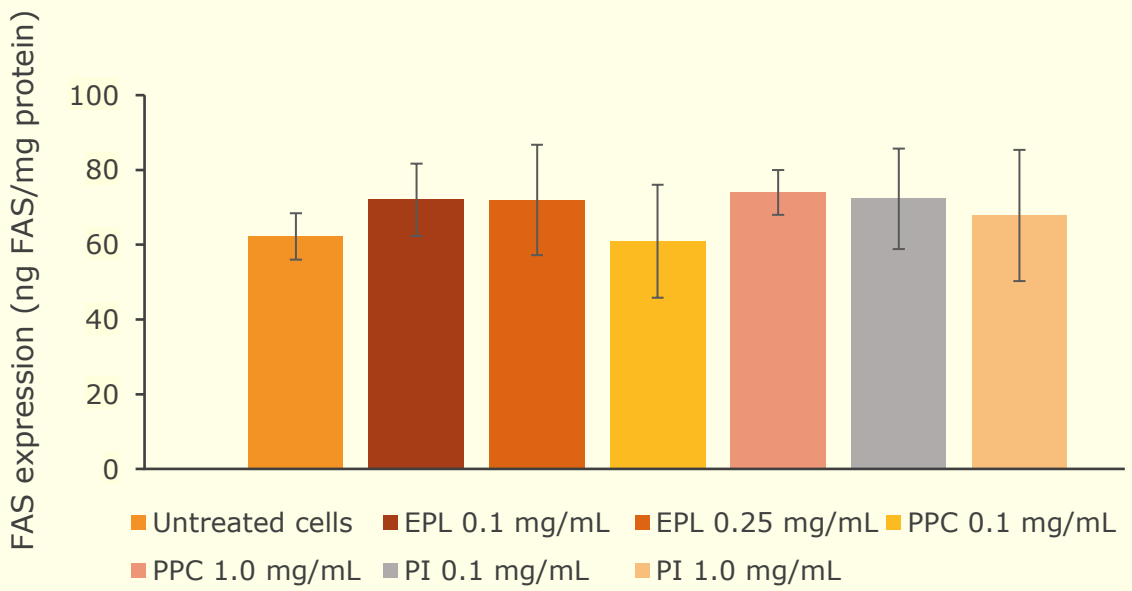
There may be an association between FAS and steatosis in human livers[1]

FAS, fatty acid synthase; NAFLD, non-alcoholic fatty liver disease

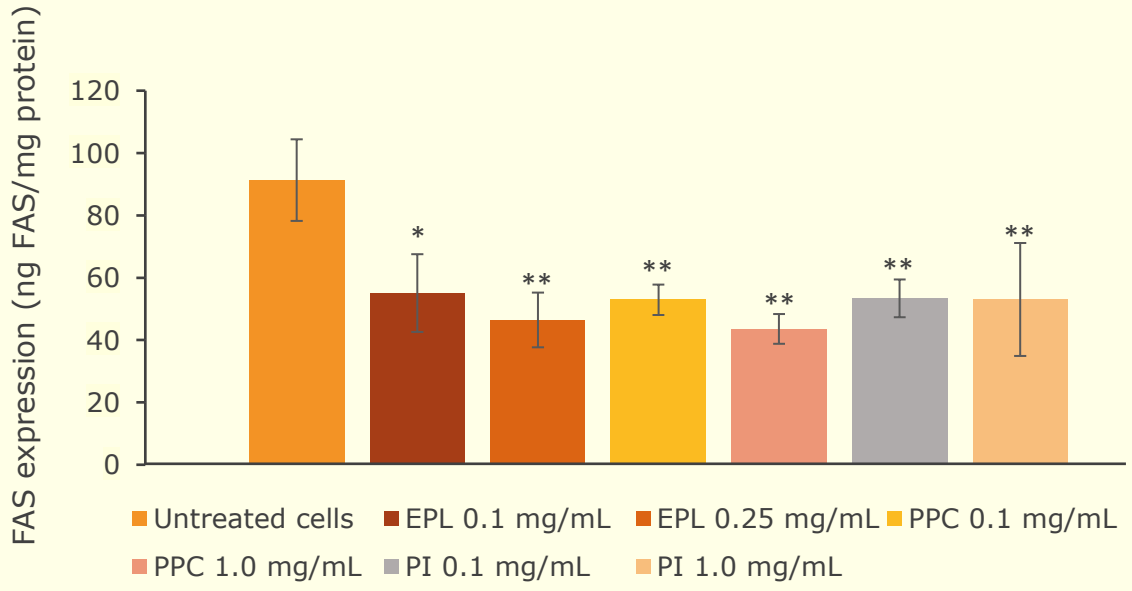
Dorn C er al. Int J Clin Exp Pathol 2010;3(5):505–14; Jensen-Urstad & Semenkovich. Biochim Biophys Acta. 2012;1821(5):747–53

FAS expression was decreased with phospholipid administration in steatotic HepaRG cells

FAS expression remained unchanged with EPL, PPC and PI treatment in LPS-treated **HepaRG** cells[1]



FAS expression decreased with EPL, PPC and PI treatment in LPS-treated **steatotic HepaRG** cells [1]



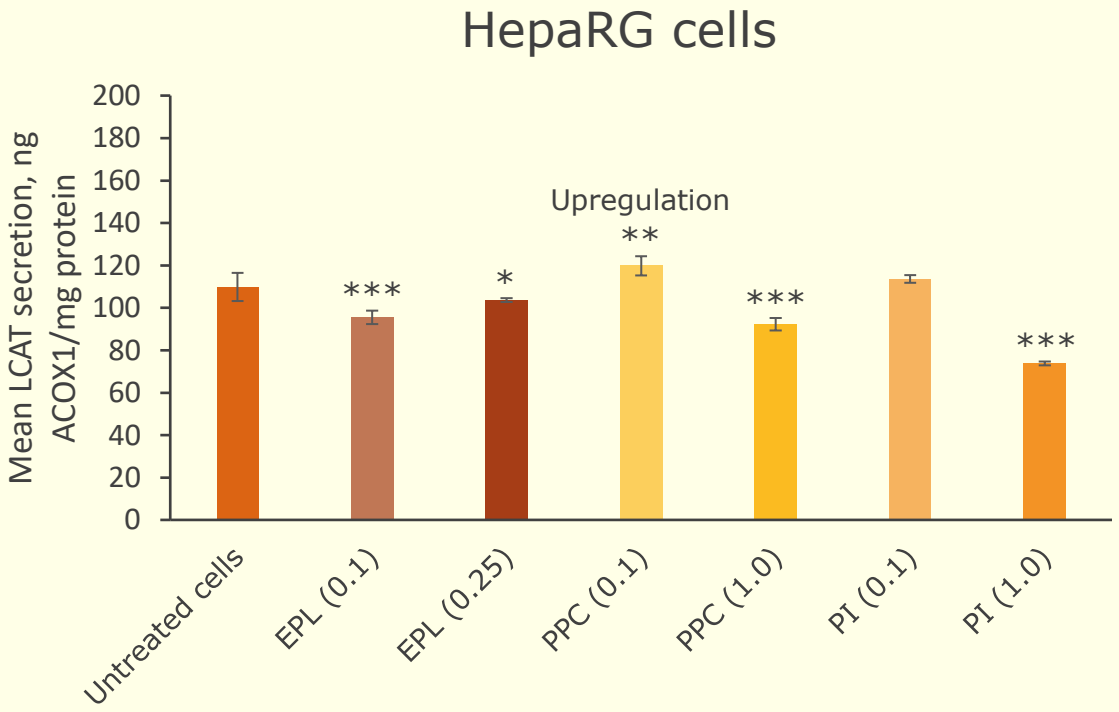
In rats with induced fatty liver, **those receiving dietary PC did not show an increase in FAS expression,** but those without dietary PC did[2]

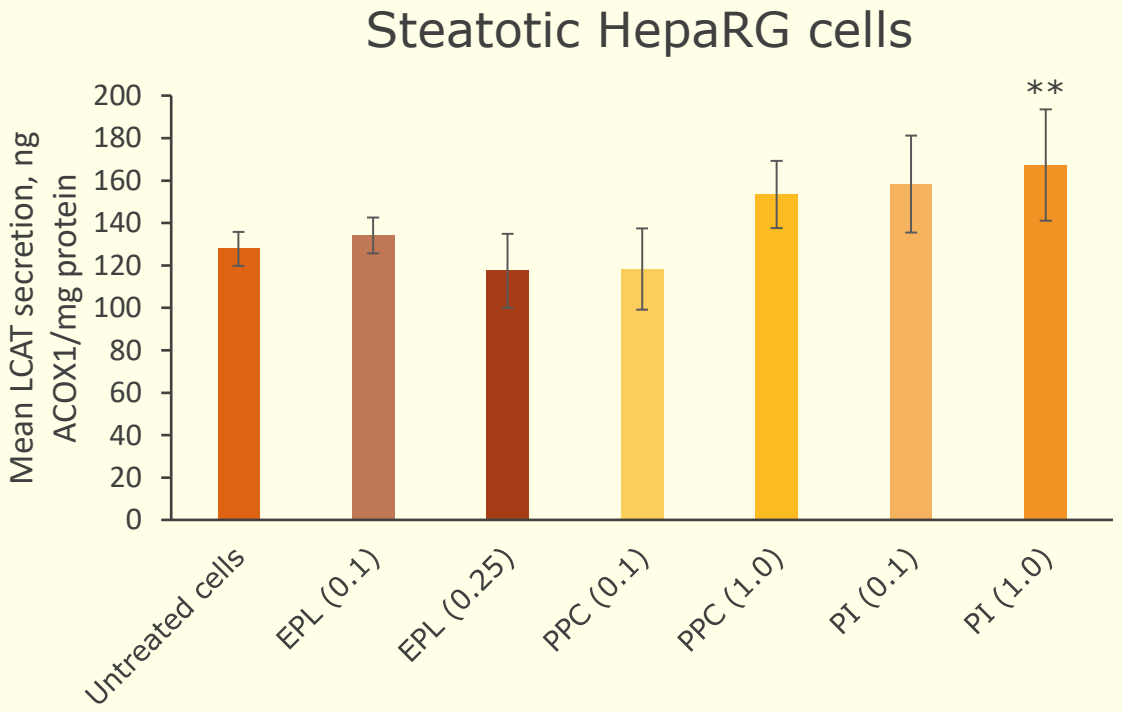
*P=0.001. **P<0.001. EPL, essential phospholipid; FAS, fatty acid synthase; LPS, lipopolysaccharide; PI, phosphatidylinositol; PC, phosphatidylcholine; PPC, polyenyl phosphatidylcholine

1. Wupperfeld D, et al. EASL. 2022; 2. Buang Y, et al. Nutrition. 2005;21:867–73.

LCAT secretion was reduced by EPL, PPC and PI treatment in LPS-treated HepaRG cells

- LCAT is involved in HDL metabolism and activity levels are elevated in patients with NAFLD[1,2]
- **In HepaRG cells, administration of EPL, PPC and PI reduced LCAT secretion**:[3]





In rats fed a hypercholesterolemic diet, those **receiving phospholipid treatment had increased LCAT activity**[4]

*p<0.05; **p<0.01; ***p<0.0001
ACOX1, acyl Coenzyme A oxidase; EPL, essential phospholipid; HDL, high-density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine

1. Calabresi L and Franceschini G. Trends Cardiovasc Med. 2010;20(2):50–3; 2. Nass KJ, et al. Eur J Clin Invest. 2018;48(9):e12988; 3. Wupperfeld D, et al. EASL. 2022; 4. Iwata T, et al. J Nutr Sci Vitaminol (Tokyo). 1993;39(1):63–71.

Summary

1. This was the first study evaluating the effects of EPL on steatotic cell lines *in vitro*
2. EPL has membrane protective and lipid regulating effects, and the study presented here provided new insights into the molecular mechanisms responsible for these properties
3. Administration of EPL, PPC and PI reduced IL-6 secretion in HepaRG cells and increased IL-6 secretion in steatotic HepaRG cells
4. *In vitro* EPL, PPC and PI modulate the activity and expression of liver metabolising enzymes including ACOX1, and LCAT but have no effect on G6PD
5. Phospholipid administration decreased FAS expression in steatotic HepaRG cells, possibly causing a reduction in hepatically-produced fatty acids

ACOX1, acyl Coenzyme A oxidase; EPL, essential phospholipid; FAS, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; IL-6, interleukin-6; LCAT, lecithin cholesterol acyltransferase; MoA, mechanism of action; PI, phosphatidylinositol; PPC, polyenyl phosphatidylcholine.