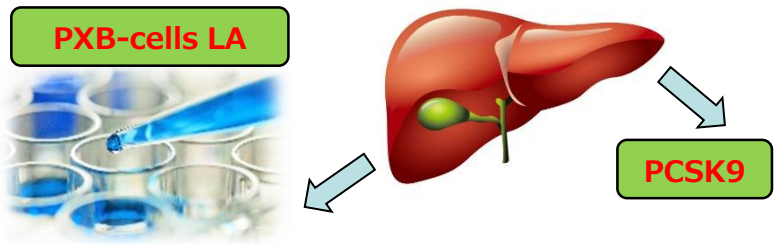
Using PXB-cells LA, Example of PCSK9 measurement -In Vitro Assay- as a fatty liver and normal liver model

- For Research Use-



PXB-cells LA (PhoenixBio Co., Ltd.)

- Fresh human hepatocytes isolated from PXB mice (PhoenixBio Inc.) (for lipid research)
- Culturable for a certain period of time
Fatty liver model or normal liver model can be selected.
- Response to drugs and other substances can be measured during the culture period.
- Cultured supernatant and cultured cells can be used.
- Production of various human-derived proteins and lipids can be measured.

PCSK9

PCSK9 degrades the LDL receptor expressed on the surface of hepatocytes, thereby reducing the liver's ability to remove LDL Cholesterol from the blood.

The PCSK9 inhibitor evolocumab blocks the binding of PCSK9 to the LDL receptor. This prevents the breakdown of the LDL receptor, resulting in a strong decrease in blood LDL Cholesterol.

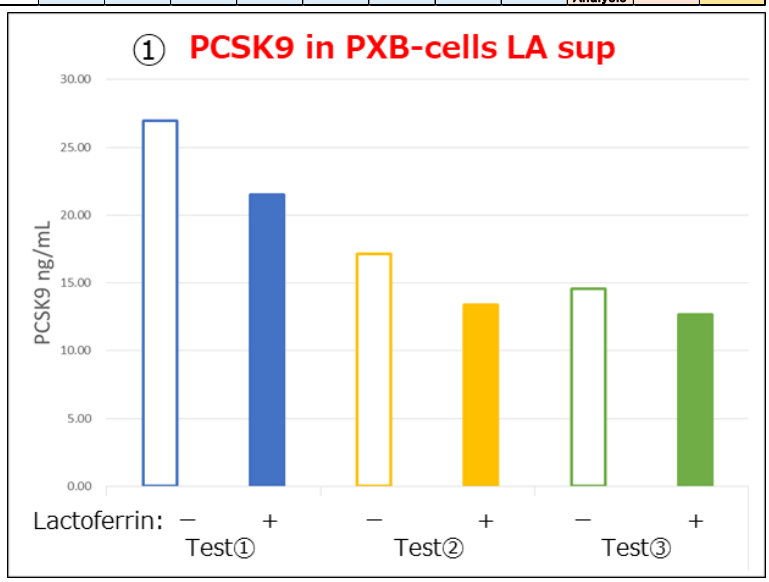
Culture of PXB-cells LA

Test① : 10 d
: Fatty Liver model
Test②／③ : 15 d／17 d
: Normal Liver model
↓
↓←add : +/－Lactoferrin (100μg/mL)
↓culture
Harvest cells and sup
↓
Measurement of PCSK9, others

Results :
① PCSK9 production decreased as the culture progressed from fatty to normal liver. In addition, PCSK9 production tended to be suppressed by the addition of lactoferrin.

Culture schedule of PXB-cells LA

Model	Test	Day7	8	9	10	11	12	13	14	15	16	17
Fatty Liver model	Test①	Medium Change	Medium Change for Analysis	⇒	Harvest							
Normal Liver model	Test②		➡	➡	Medium Change	➡	➡	Medium Change for Analysis	⇒	Harvest		
	Test③		➡	➡	Medium Change	➡	➡	Medium Change	➡	Medium Change for Analysis	⇒	Harvest



Immuno-Biological Laboratories Co. Ltd. TEL: 0274-50-8666 Email: do-ibl@ibl-japan.co.jp URL: www.ibl-japan.co.jp

Using PXB-cells LA, Measurement of PCSK9 related factors -In Vitro Assay- as a fatty liver and normal liver model

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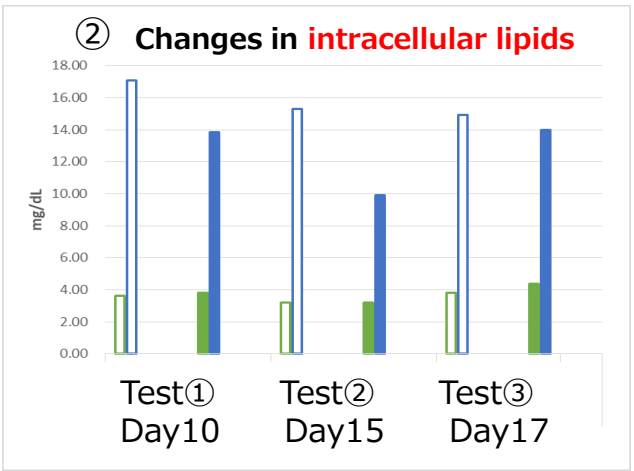
Changes in intracellular lipids and lipid metabolism-related factors

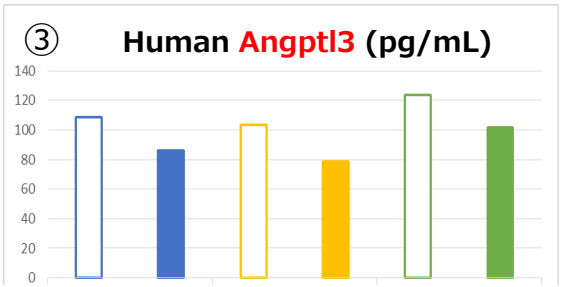


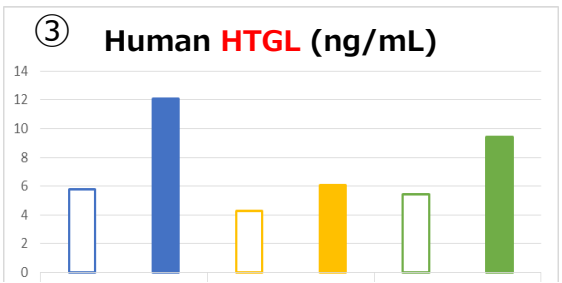
② Although TG decreased slightly with the course of culture, no significant change was observed in Cholesterol. Lactoferrin reduced TG levels in each test.

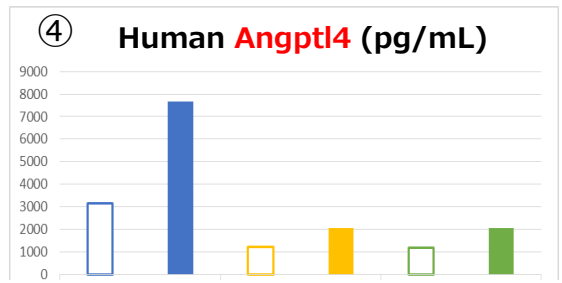
③ It was suggested that lactoferrin suppresses the expression of Angptl3, which negatively regulates LPL activity, and increases HTGL, thereby suppressing intracellular TG production and reducing lipid secretion.

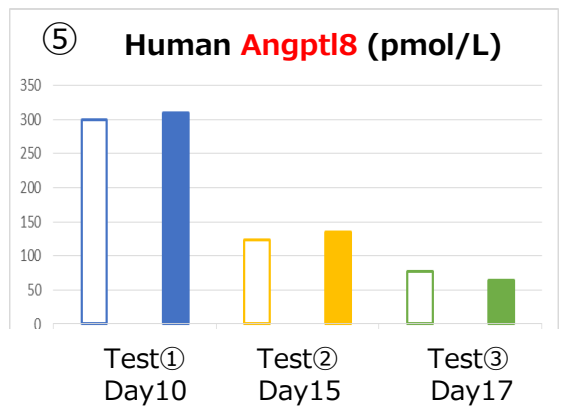
④ Angptl4 production was increased by the addition of lactoferrin, especially in the fatty liver model.

⑤ The amount of Angptl8 producion was decreased as the culture progressed, but the addition of lactoferrin had little impact.









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Measurement of Cholesterol/TG concentration and Lipoprotein particle number in culture sup of PXB-cells LA, by LipoSEARCH

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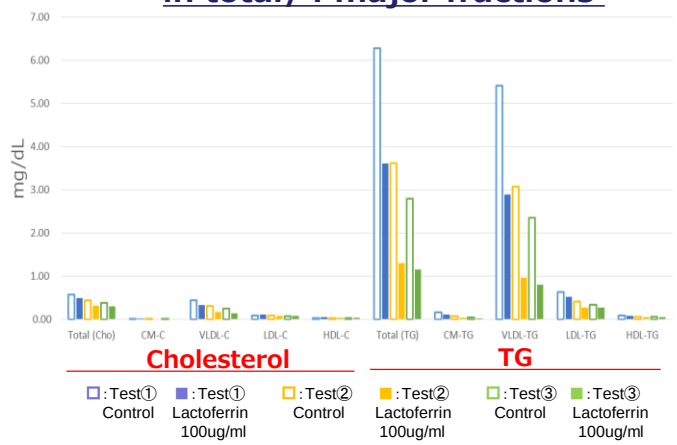
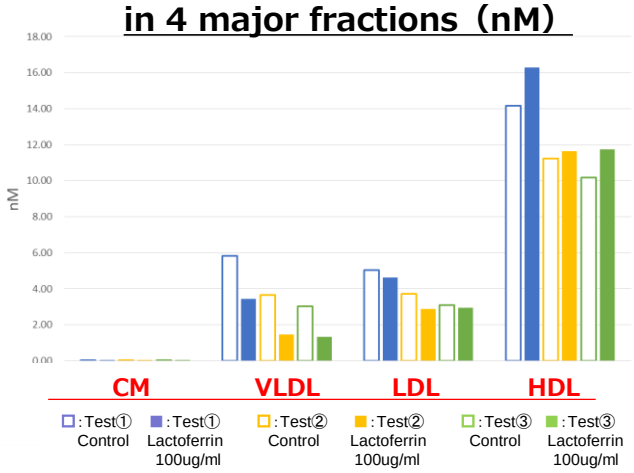
①Cholesterol/TG conc.(mg/dL) in total/4 major fractions



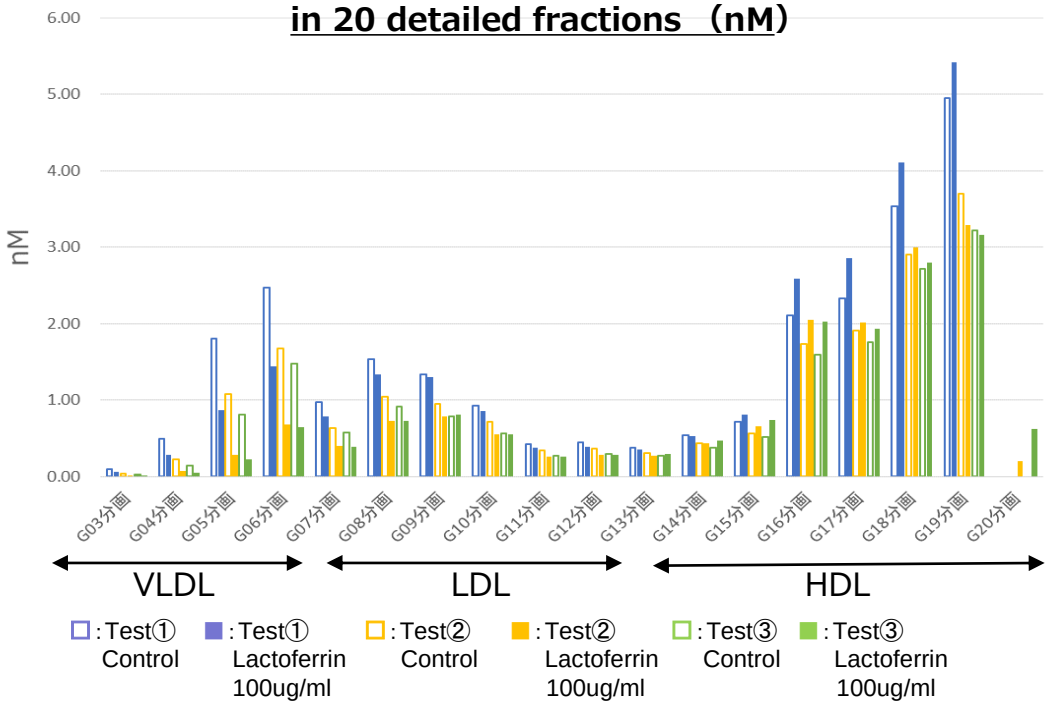
②Lipoprotein Particle Numbers in 4 major fractions（nM）



① Lipids secreted into the culture supernatant were mainly VLDL lipids. Cholesterol and TG tendded to decrease as the culture progresses. Addition of lactoferrin also reduced lipids in each test.

② HDL with low lipid content can be evaluated more easily by analyzing the number of particles. Addition of lactoferrin reduced the secretion of VLDL and LDL, but the secretion of HDL was increased in fatty liver.

③ Lipoprotein Particle Numbers in 20 detailed fractions（nM）



③ In test ① (fatty liver model), there was an increasing tendency in the HDL fraction, especially small HDL particles (G16 - G19 fraction) increased. Furthermore, addition of lactoferrin increased their secretion.

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Advantages of this measurement system
−PXB-cells LA&LipoSEARCH−

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Advantages of PXB-cells LA

- Fresh human hepatocytes, isolated from PXB mice, homogenous and less lot variation
- Can be cultured for a certain period of time, during which time responses to drugs can be measured
- Fatty liver model or normal liver model can be selected depending on the culture period
- Culture supernatant and cultured cells can be used
- The production of various human-derived proteins and lipids can be measured
- *In vitro* cell culture system that simplifies in vivo phenomena

Advantages of LipoSEARCH

- Highly sensitive lipoprotein detailed analysis service
- No sample pretreatment required
- Can measure even low-concentration samples such as culture supernatant
- Comprehensive evaluation is possible (Total cholesterol, total TG, major 4 fractions, subclass, number of particles, etc.)
- Can identify lipid components involved in reactivity to drugs

Advantages of PXB-cells LA&LipoSEARCH

- Using a simple and reproducible in vitro human hepatocyte culture system, it is possible to elucidate the factors involved in lipid metabolism and lipid dynamics in the human liver.

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