BPB Reports

Report

An *in Vitro* System for Screening Insulin-Sensitizing Agents: Leveraging Human Hepatocyte Models of MASLD and FGF21 Response

Masaki Takahashi,[a] Sayaka Tomatsu,[b] Mutsumi Inamatsu,[a] Nami Yoshikawa,[c] Chise Tateno,[a,d,e] Keishi Hata,[b] and Masakazu Kakuni[f,*]

[a]Department of PXB-mouse Production, PhoenixBio Co., Ltd., 3-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-0046, Japan; [b]Brewing Research Station, Akita Research Institute of Food and Brewing, 4-26 Sanuki, Araya-machi, Akita 010-1623, Japan; [c]Department of Study Service, PhoenixBio Co., Ltd., 3-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-0046, Japan; [d]Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, Hiroshima, 734-8553, Japan; [e]Wakayama Medical University, 811-1 Kimiidera, Wakayama-shi 641-8509, Japan; [f]Department of Business Development, PhoenixBio Co., Ltd., 3-4-1 Kagamiyama, Higashihiroshima, Hiroshima 739-0046, Japan

Received February 6, 2026; Accepted April 15, 2026

Progression of metabolic dysfunction-associated steatotic liver disease leads to insulin resistance, a condition driven by the induction of specific hepatokines. We previously developed an *in vitro* steatotic liver model, PXB-cells LA (Lipid Analysis), derived from fresh human hepatocytes (PXB-cells®) isolated from humanized mouse livers. In the present study, we investigated its utility as a screening system for insulin-sensitizing agents. Our results demonstrated that metformin and rosiglitazone, well-known treatments for type 2 diabetes, reduced the expression of the prodiabetic hepatokines leukocyte cell-derived chemotaxin 2 and fetuin-A in PXB-cells LA. Furthermore, while fibroblast growth factor 21 enhances systemic insulin sensitivity, both agents upregulated fibroblast growth factor 21 production at both the mRNA and protein levels in this model.

Key words PXB-cells LA, human primary hepatocyte, insulin resistance, hepatokine, fibroblast growth factor 21

INTRODUCTION

The global prevalence of Western-style diets, characterized by an excessive intake of carbohydrates and fats, has contributed to the global increase in metabolic syndrome. Central to this health crisis is metabolic dysfunction-associated steatotic liver disease (MASLD), which is defined as hepatic lipid accumulation concomitant with obesity or type 2 diabetes. When accompanied by inflammation and fibrosis, MASLD progresses to metabolic dysfunction-associated steatohepatitis, significantly increasing the risk of cirrhosis and hepatocellular carcinoma.[1)]

Emerging evidence suggests that the liver is a pivotal endocrine organ that regulates systemic metabolism by secreting hepatokines. During MASLD progression, hepatocyte stress triggers the excessive release of prodiabetic hepatokines, such as leukocyte cell-derived chemotaxin 2 (LECT2).[2)] These proteins circulate systemically and impair insulin signaling in peripheral tissues, including skeletal muscle and adipose tissue. This exacerbates systemic insulin resistance, which in turn increases the flux of free fatty acids from the adipose tissue back to the liver, creating a vicious cycle that accelerates hepatic injury. In contrast, the liver secretes protective hepatokines, most notably, fibroblast growth factor 21 (FGF21). FGF21 improves glucose and lipid metabolism, exerts antiobesity effects, and enhances insulin sensitivity.[3)] Consequently, targeting the balance between the deleterious and protective hepatokines has emerged as a promising therapeutic strategy (Fig. 1A).

Given the clinical need for physiologically relevant models to study this complex pathology, we developed the PXB-cells LA (Lipid Analysis) model[4)].-This model is derived from PXB-cells—fresh human hepatocytes from chimeric mice with humanized livers—and established using a lipid-maintained medium. Although PXB-cells are naturally rich in lipid droplets, these droplets rapidly disappear in standard *in vitro* culture. The lipid-maintained approach preserves intracellular lipids and allows the model to reproduce key MASLD features, including lipid accumulation, mitochondrial dysfunction, and elevated inflammatory markers.

In this study, we proposed a novel screening system for insulin-sensitizing agents that quantifies hepatokine secretion within the PXB-cells LA platform. Furthermore, we established a comprehensive evaluation system for metabolic syndrome drug candidates by integrating existing assays to assess lipoprotein and/or cholesterol uptake capacity.

*To whom correspondence should be addressed. e-mail: masakazu.kakuni@phoenixbio.co.jp

© 2026 Author(s) BPB Reports applies the Creative Commons Attribution (CCBY) license to works we published. The license was developed to facilitate open access - namely, free immediate access to, and unrestricted reuse of, original works to all types. Under this license, authors agree to make articles legally available for reuse, without permissions of fees, for virtually any purpose. Anyone may copy, distribute, or reuse these articles, as long as the author and original source are properly cited. https://creativecommons.org/licenses/by/4.0/

MATERIALS AND METHODS

Culture of Hepatocytes Human hepatocytes (PXB-cells) were isolated from humanized murine livers 14–16 weeks post-transplantation.[5] PXB-cells LA, which are MASLD-like hepatocytes derived from PXB-cells, were then prepared following the procedure previously described[4] (Fig. 1B). On Day 8 of culture, the PXB-cells LA were treated with either 1.0 mM metformin (an AMP-activated protein kinase activator; Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) or 10 μM rosiglitazone (a peroxisome proliferator-activated receptor-γ agonist; Tokyo Chemical Industry Co., LTD., Chuo-ku, Tokyo, Japan), or left untreated for 2 days. These agents were assayed at concentrations based on previously established literatures.[6,7] The culture medium was subsequently subjected to a lipoprotein profile assay (LipoSEARCH®; Immuno-Biological Laboratories, Fujioka, Gumma, Japan) or ELISA, while the cells were harvested for mRNA isolation.

Real-Time Polymerase Chain Reaction (RT-PCR) Total RNA was isolated using QuickGene RNA Cultured Cell Kit S (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). Template cDNA synthesis was performed with 5 μg of total RNA using the PrimeScript RT reagent Kit (Takara Bio, Kusatsu, Shiga, Japan). In a fluorescent temperature cycler (CFX Connect™ RT-PCR Detection System; Bio-Rad Laboratories, Hercules, CA, USA), 2.5% of each RT reaction solution was amplified in 25 μL of 1 × SYBR Premix Ex Taq (Takara Bio) containing 0.2 μM of each primer. The samples were incubated in a thermal cycler for initial denaturation at 95°C for 10 s, followed by 40 cycles of PCR. Each cycle consisted of 95°C for 5 s and 60°C for 30 s. The relative expression of each mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase. The following primers were used for PCR amplification: FGF21-forward (5′-GGGAGTCAAGACATCCAGGT-3′) and FGF21-reverse (5′-GGCTTCGGACTGGTAAACAT-3′), glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-forward (5′-GCACCGTCAAGGCTGAGAAC-3′) and GAPDH-reverse (5′-TGGTGAAGACGCCAGTGGA-3′), fetuin-A-forward (5′-TCCTTGGGGATACAAACACACC-3′) and fetuin-A-reverse (5′-TACCACGGAAAACTTGCCATC-3′), LECT2-forward (5′-GTGTTCGAATATCTGGAAGAGGT-3′) and LECT2-reverse (5′- AAGGGCAATAGAGTTCCAAGT-3′), selenoprotein P-forward (5′-TCATCAAGGAATCTCTTCTCG-3′) and selenoprotein P-reverse (5′-CAAGACGGCCACATCTATCA-3′).

Enzyme-Linked Immunosorbent Assay (ELISA) Albumin levels in the culture medium were quantified using the LZ-Test "Eiken" U-ALB (Eiken Chemical, Taito-ku, Tokyo, Japan). Human total angiotensinogen, apolipoprotein A1 (APOA1), APOA5, APOB100, FGF21, and hepatic triglyceride lipase (HTGL) levels were measured using specific ELISA kits (ImmunoBiological Laboratories) according to the manufacturer's instructions. The amount of each hepatic protein in the culture medium was normalized to total intracellular protein content.

Statistical Analysis Data are expressed as the mean ± standard deviation. Statistical analyses were performed using BellCurve for Excel (Social Survey Research Information Co.,

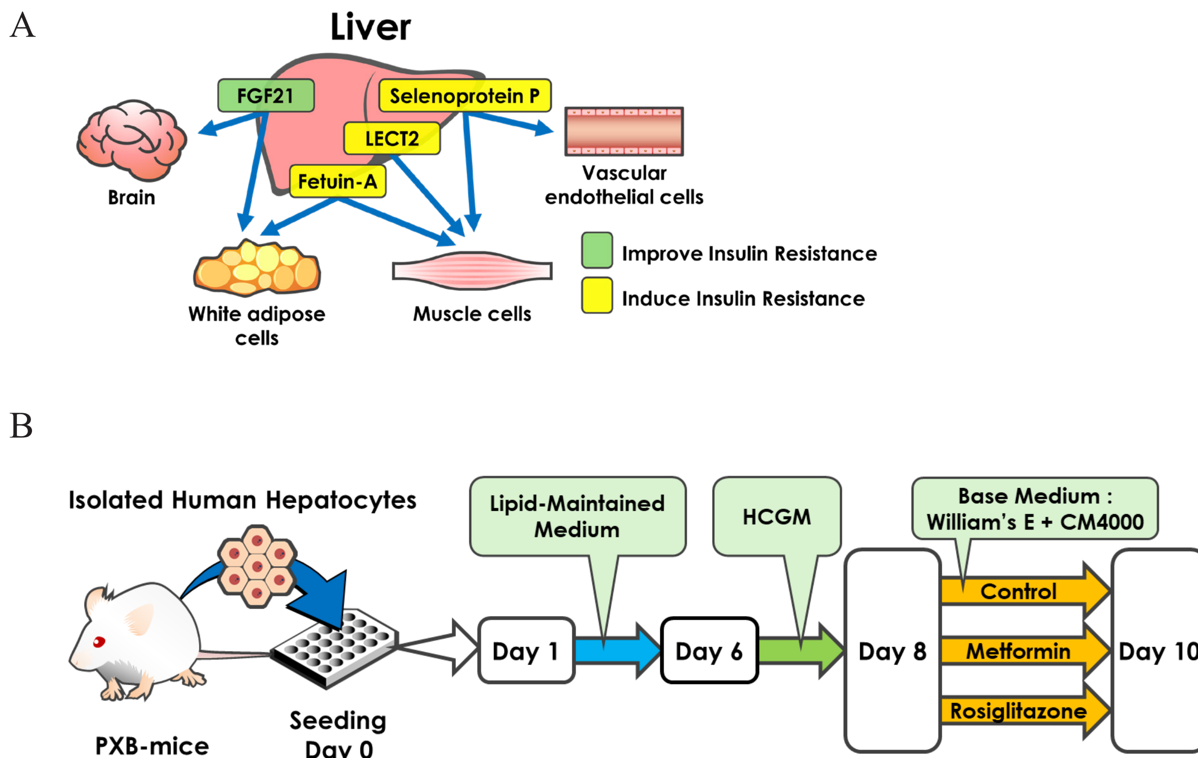


Fig. 1. Promising Therapeutic Strategies Targeting Hepatokines Associated with Fatty Liver Progression (A) and the Preparation Protocol for PXB-Cells LA (B).

A: Therapeutic strategies targeting hepatokines: Strategies include upregulation of FGF21 (fibroblast growth factor 21) and downregulation of selenoprotein P, LECT2 (leukocyte cell-derived chemotaxin 2), and fetuin-A.

B: Preparation protocol for PXB-cells LA: PXB-cells were seeded at a density of 4 × 10^5 cells/well in collagen-coated 24-well microplates (Day 0). The cells were cultured in Lipid-Maintained Medium (Phoenix Bio, Higashi-Hiroshima, Japan) from seeding until Day 6, followed by maintenance in hepatocyte growth medium (HCGM), a hepatocyte functional maintenance medium for PXB-cells, for 2 days.[4,5]

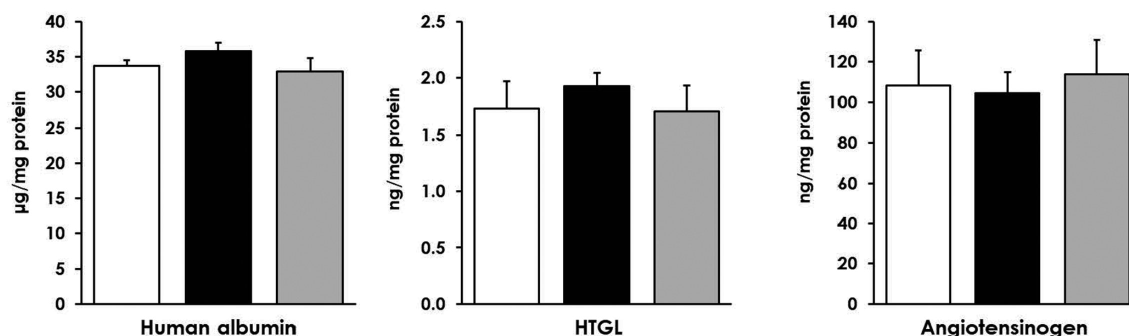


Fig. 2. Effects of Metformin and Rosiglitazone on Hepatic Markers in PXB-Cells LA.

PXB-cells LA were treated with 1.0 mM metformin (black bars), 10 µM rosiglitazone (gray bars), or vehicle control (white bars) for 2 days. Levels of three hepatic markers in the culture medium were determined using ELISA. HTGL: hepatic triglyceride lipase. Data are expressed as the mean ± standard deviation (*n* = 4, *P < 0.05 vs untreated group).

Table 1. Quantity of Cholesterol and Triglyceride in Each Lipoprotein Fraction Secreted from PXB-cells LA.

	Cholesterol (µg/mg protein)			Triglycerides (µg/mg protein)		
	untreated	metformin	rosiglitazone	untreated	metformin	rosiglitazone
Total	4.3 ± 0.2	3.4 ± 0.2*	4.0 ± 0.3	53.3 ± 2.2	40.1 ± 2.2*	51.6 ± 3.9
VLDL						
all	3.7 ± 0.2	2.9 ± 0.2*	3.5 ± 0.3	49.5 ± 2.1	37.3 ± 2.0*	47.9 ± 3.6
large	2.9 ± 0.1	2.3 ± 0.1*	2.8 ± 0.2	40.6 ± 1.9	30.9 ± 1.8*	39.5 ± 3.1
medium	0.6	0.5*	0.6 ± 0.1	7.5 ± 0.3	5.3 ± 0.2*	6.9 ± 0.5
small	0.2	0.1	0.2	1.5 ± 0.1	1.1 ± 0.1*	1.5 ± 0.1
LDL						
all	0.4	0.3*	0.4	3.2 ± 0.1	2.4 ± 0.1*	3.2 ± 0.2
large	0.2	0.1	0.1	1.5 ± 0.1	1.1 ± 0.1*	1.5 ± 0.1
medium	0.1	0.1	0.1	0.8	0.7*	0.9 ± 0.1
small	0.1	0.1	0.1	0.5	0.3*	0.5
very small	0.1	0.1	0.1	0.4	0.3*	0.4
HDL						
all	0.2	0.1	0.2	0.6	0.4*	0.5 ± 0.1
very large	0.1	0	0.1	0.1	0.1	0.1
large	0	0	0	0.1	0.1	0.1
small	0	0	0	0.1	0.1	0.1
very small	0	0	0	0.2	0.2	0.2

Data are expressed as the mean ± standard deviation (*n* = 4, *P < 0.05 vs untreated group).

Ltd., Shinjuku-ku, Tokyo, Japan). Significant differences were analyzed using the Kruskal-Wallis test, followed by Steel's post-hoc test. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

We aimed to establish an evaluation system for the production of insulin resistance-related hepatokines using PXB-cells LA, a human steatotic liver injury cell model. This system was validated using the known pharmacological actions of two insulin sensitizers: metformin and rosiglitazone. Before investigating the pharmacological effects of both agents in the PXB-cells LA model, we confirmed the absence of potential hepatic damage by monitoring liver function markers, including albumin, HTGL, and angiotensinogen. While albumin and HTGL levels typically decrease as liver damage progresses, cirrhotic livers maintain near-normal plasma angiotensinogen levels until the final stages of hepatic insufficiency.[8-10] In our study, 1.0 mM metformin and 10 µM rosiglitazone did not alter these markers, suggesting no significant hepatic damage in the PXB-cells LA model (Fig. 2).

Metformin and rosiglitazone have been shown to normalize lipid, APOA5, and/or APOB100 *in vivo* or *in vitro*.[11-13] We investigated whether these agents affected the lipoprotein profile of PXB-cells LA.

Our results demonstrated that metformin reduced cholesterol and triglyceride levels across all lipoprotein fractions; however, rosiglitazone treatment showed no significant changes compared with the untreated control (Table 1). These findings indicate that rosiglitazone does not influence lipoprotein secretion or lipid loading onto lipoproteins under the present experimental conditions. Nonetheless, further studies will be valuable to determine whether rosiglitazone affects these processes under different experimental conditions.

Furthermore, we measured the levels of APOA5 and APOB100, the primary apolipoproteins in very-low-density lipoproteins (VLDL) and low-density lipoproteins, respectively. As shown in Fig. 3, metformin downregulated extracellular APOA5 and APOB100 in PXB-cells LA, whereas APOA1 levels remained unaffected. These findings indicate that metform-

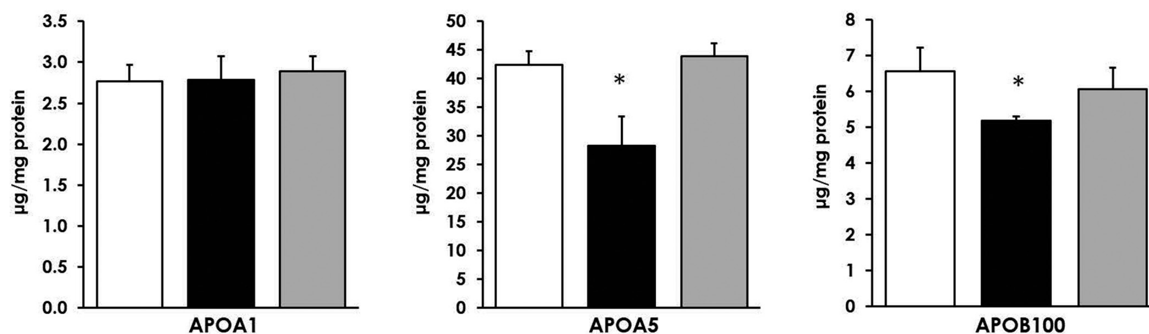
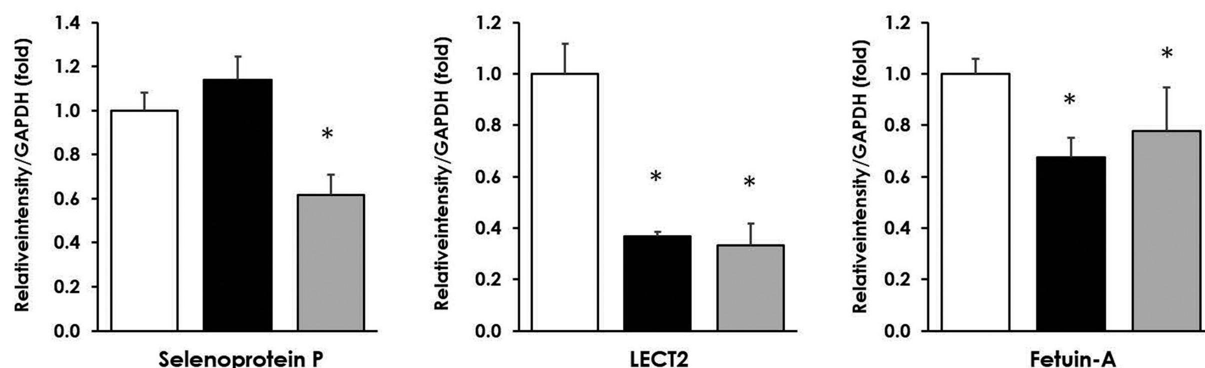


Fig. 3. Effects of Two Insulin-Sensitizing Agents on Apolipoprotein Production in PXB-Cells LA.

PXB-cells LA were treated with 1.0 mM metformin (black bars), 10 μM rosiglitazone (gray bars), or vehicle control (white bars) for 2 days. The levels of the three apolipoproteins in the culture medium were determined using ELISA. APOA1: Apolipoprotein A1, APOA5: Apolipoprotein A5, APOB100: Apolipoprotein B100. Data are expressed as the mean ± standard deviation ($n = 4$, $*P < 0.05$ vs untreated group).

A



B

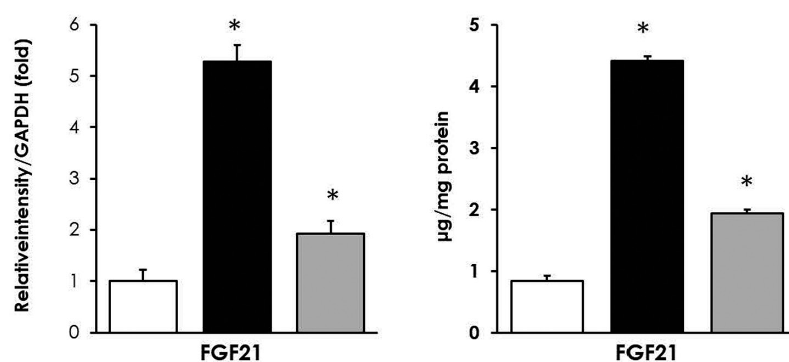


Fig. 4. Hepatokine Profiling in PXB-Cells LA.

PXB-cells LA were treated with 1.0 mM metformin (black bars), 10 μM rosiglitazone (gray bars), or vehicle control (white bars) for 2 days. Hepatokine gene expression was analyzed using real-time RT-PCR, and protein levels in the culture medium were quantified using ELISA. LECT2: leukocyte cell-derived chemotaxin 2, FGF21: fibroblast growth factor 21. Data are expressed as the mean ± standard deviation ($n = 4$, $*P < 0.05$ vs untreated group).

in (1.0 mM) can modulate lipoprotein production in a manner consistent with previously reported clinical findings, without inducing hepatic damage. Based on these results, we decided to use both agents at this concentration for subsequent experiments to evaluate their effects on fatty liver-induced hepatokines in PXB-cells LA.

Previous studies have shown that metformin and rosiglitazone can regulate hepatokine expression. Generally, these insulin-sensitizing agents suppress hepatokines that induce insulin resistance and promote those that improve it.[6,14,15] In the present study, RT-PCR analysis indicated that both treatments significantly downregulated several hepatokine genes, except *selenoprotein P* in the metformin group (Fig. 4A). Furthermore, we investigated whether these agents modulate FGF21 levels. Metformin upregulated FGF21 expression by 5.3-fold at both the mRNA and protein levels, whereas rosigl-

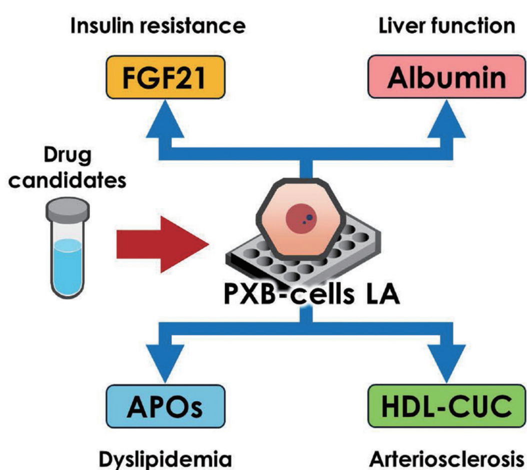


Fig. 5. Comprehensive Evaluation System for Metabolic Syndrome Drug Candidates Using PXB-Cells LA.

APOs: Apolipoproteins, HDL-CUC: High-density lipoprotein cholesterol uptake capacity.

itazone induced a 1.9-fold and 2.3-fold increase, respectively (Fig. 4B). These findings are largely consistent with those of previous reports,[16,17] suggesting that the PXB-cells LA culture system serves as a suitable platform for screening the insulin-sensitizing activities of drug candidates.

We have previously developed a high-throughput screening assay for antilipidemic agents by measuring APOA5 and APOB100 levels in PXB-cells culture media via ELISA.[7] In the present study, we demonstrated that measuring APOA5 (a constituent of VLDL) and APOB100 (a constituent of VLDL/low-density lipoproteins) levels can effectively detect reductions in lipoprotein secretion. Based on these findings, we showed that PXB-cells LA culture media can be used to evaluate high-density lipoprotein cholesterol uptake capacity (HDL-CUC), a functional index reflecting the ability of HDL to retrieve excess cholesterol from peripheral tissues for transport to the liver.[18]

Furthermore, we demonstrated the efficacy of this assay system in evaluating insulin resistance improvers, using FGF21 expression as an indicator. This platform also enables the simultaneous evaluation of drug-induced hepatic injury via specific liver function markers. Nonetheless, the present study evaluated only two insulin-sensitizing agents, and the applicability of this assay system to drug classes with distinct mechanisms of action—such as glucagon-like peptide-1 receptor agonists and sodium–glucose cotransporter 2 inhibitors—remains to be unclear. Assessing these therapeutic classes will be an important direction for future research.

Consequently, we anticipate that PXB-cells LA will serve as a valuable tool for analyzing metabolic disorders originating from fatty liver and for developing novel therapeutic agents (Fig. 5).

Conflict of interest Masaki Takahashi, Mutsumi Inamatsu, Nami Yoshikawa, Chise Tateno, and Masakazu Kakuni are employees of PhoenixBio Co., Ltd.

REFERENCES

1) Kawaguchi T. New Concept of Fatty Liver: metabolic dysfunction-associated fatty liver disease. *Kanzo*, **64**, 33–43 (2023).
2) Misu H. Identification of hepatokines involved in pathology of type 2 diabetes and obesity. *Endocr. J.*, **66**, 659–662 (2019).
3) Ge X, Wang Y, Lam KSL, Xu A. Metabolic actions of FGF21: molecular mechanisms and therapeutic implications. *Acta Pharm. Sin. B*, **2**, 350–357 (2012).
4) Takahashi M, Tomatsu S, Inamatsu M, Yoshikawa N, Hata K, Kakuni M. The evaluation of lipid analysis for PXB-Cells LA as a human non-alcoholic fatty liver disease model. *BPB Reports*, **7**, 147–156 (2024).
5) Yamasaki C., Ishida Y., Yanagi A., Yoshizane Y., Kojima Y., Ogawa Y., Kageyama Y., Iwasaki Y., Ishida S., Chayama K., Tateno C. Culture density contributes to hepatic functions of fresh human hepatocytes isolated from chimeric mice with humanized livers: Novel, long-term, functional two-dimensional *in vitro* tool for developing new drugs. PLoS One, Sep 11;15(9), (2020).
6) Nygaard EB, Vienberg SG, Ørskov C, Hansen HS, Andersen B. Metformin stimulates FGF21 expression in primary hepatocytes. *Exp. Diabetes Res.*, **2012**, 465282 (2012).
7) Tomatsu S, Takahashi M, Toshima G, Hata K, Kakuni M. High-throughput screening of anti-lipidemic agents using PXB-cells®, human primary hepatocytes from humanized mice livers: assessment of lipoproteins by an enzyme-linked immunosolvent assay on apolipoproteins. *J. Biol. Macromol.*, **21**, 89–92 (2021).
8) Bernardi M, Angeli P, Claria J, Moreau R, Gines P, Jalan R, Caraceni P, Fernandez J, Gerbes AL, O'Brien AJ, Trebicka J, Thevenot T, Arroyo V. Albumin in decompensated cirrhosis: new concepts and perspectives. *Gut*, **69**, 1127–1138 (2020).
9) Fujiwara S, Yukawa S, Fujino K, Tsujimoto H, Maeshima E, Tone Y, Hasegawa S, Kawano K, Mimura K, Maeda T, Nomoto H. Enzyme-linked immunoassay of hepatic triglyceride lipase in chronic liver disease. *Kanzo*, **33**, 219–224 (1992).
10) Sansoè G, Aragno M, Wong F. Pathways of hepatic and renal damage through non-classical activation of the renin-angiotensin system in chronic liver disease. *Liver Int.*, **40**, 18–31 (2020).
11) Li R, Chen LZ, Zhao W, Zhao SP, Huang XS. Metformin ameliorates obesity-associated hypertriglyceridemia in mice partly through the apolipoprotein A5 pathway. *Biochem. Biophys. Res. Commun.*, **478**, 1173–1178 (2016).
12) Wanninger J, Neumeier M, Weigert J, Liebisch G, Weiss TS, Schäffler A, Aslanidis C, Schmitz G, Schölmerich J, Buechler C. Metformin reduces cellular lysophosphatidylcholine and thereby may lower apolipoprotein B secretion in primary human hepatocytes. *Biochim. Biophys. Acta*, **1781**, 321–325 (2008).
13) Hung YJ, Hsieh CH, Pei D, Kuo SW, Lee JT, Wu LY, He CT, Lee CH, Fan SC, Sheu WH. Rosiglitazone improves insulin sensitivity and glucose tolerance in subjects with impaired glucose tolerance. *Clin. Endocrinol. (Oxf.)*, **62**, 85–91 (2005).
14) Haukeland JW, Dahl TB, Yndestad A, Gladhaug IP, Løberg EM, Haaland T, Konopski Z, Wium C, Aasheim ET, Johansen OE, Aukrust P, Halvorsen B, Birkeland KI. Fetuin A in nonalcoholic fatty liver disease: *in vivo* and *in vitro* studies. *Eur. J. Endocrinol.*, **166**, 503–510 (2012).
15) Ochi A, Mori K, Emoto M, Nakatani S, Morioka T, Motoyama K, Fukumoto S, Imanishi Y, Koyama H, Ishimura E, Inaba M. Direct inhibitory effects of pioglitazone on hepatic fetuin-A expression. *PLoS One*, **9**, e88704 (2014).
16) Al-Kuraishy HM, Al-Gareeb AI, Saad HM, Batiha GE. The potential effect of metformin on fibroblast growth factor 21 in type 2 diabetes mellitus (T2DM). *Inflammopharmacology*, **31**, 1751–1760 (2023).
17) Zhang H, Zhu R, Sun Q, Du L. Research progress on the role of FGF21 in insulin resistance. *Front. Endocrinol. (Lausanne)*, **16**, 1619462 (2025).
18) Hata K, Okada T, Takahashi M, Arimatsu Y, Kawahira J, Murakami K, Harada A, Yoshikawa N, Inamatsu M, Kakuni M. Cholesterol uptake capacity of HDL in culture medium of fresh primary human hepatocytes: an *in vitro* system for screening anti-atherosclerosis drugs focused on HDL functions. *BMC Res. Notes*, **18**, 480 (2025).