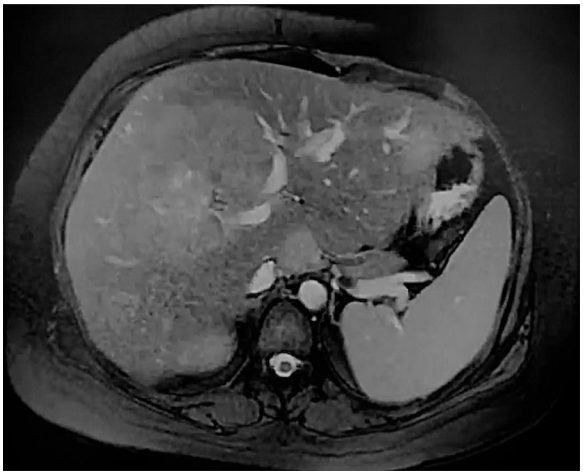

Annex 2. Simple magnetic resonance imaging

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Pirfenidone Prevents Myocarditis by Restoring Metabolic Hormone Levels in a Mouse MASH Model and its Effect on H9c2 Myoblast Viability under Glucolipotoxicity

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Introduction and Objectives: Obesity, global epidemic, can cause metabolic dysfunction-associated steatohepatitis (MASH) and cardiovascular diseases. Pirfenidone (PFD) has anti-inflammatory and antifibrotic properties. We investigated the effects of PFD on metabolic hormones expression and myocarditis in a mouse MASH model and its effect on H9c2 cells viability under glucolipotoxicity.

Materials and Patients: Twenty-week-old male C57BL/6J mice were divided into two groups: one group was fed a normal diet (ND, 3.1 kcal/g plus normal water, n=7), while the other group was fed a high-fat, high-carbohydrate diet (HFHC, 5.1 kcal/g plus water containing 2.31% fructose, 1.89% sucrose; n=14) for 16 weeks. At week 8, seven HFHC mice were administered PFD at a dosage of 300 mg/kg/day by gavage. Insulin tolerance tests (ITT), dry chemistry analysis, ELISA, histological staining (Hematoxylin-Eosin and Masson's Trichrome), and morphometric analyzes of the tissues were evaluated. H9c2 cells were treated with the following concentrations: 100 μM, 200 μM, 400 μM PA (PA), 15 mM, 30 mM glucose, and 0.3 mM, 0.5 mM, 1 mM 1.5 mM PFD. H9c2 cells viability under glucolipotoxicity were evaluated by MTT assay and Oil red O staining. The data were analyzed using one-way ANOVA followed by Tukey's post-hoc test in Graphpad Prism v10.0.

Results: HFHC mice developed MASH, myocarditis and fibrosis ($P \leq 0.05$). Additionally, resistin and AST levels significantly increased ($P \leq 0.05$). PFD prevented elevated parameters in HFHC mice ($P \leq 0.05$), such as body weight, epididymal fat weight, liver weight and heart weight; including body weight/tibia length ratio, heart weight/tibia length ratio and epididymal fat weight/tibia length ratio; hormone levels: insulin, glucagon, leptin, and plasminogen activator inhibitor-1 (PAI-1); lipid profile: total cholesterol, triglycerides, LDL, and VLDL; adipocyte hypertrophy, inflammatory foci, and fibrosis in liver and cardiac tissues. Additionally, PFD reduced ALT expression and tibia length ($P \leq 0.05$). The heart weight/body weight ratio decreased in HFHC mice ($P \leq 0.05$), PFD recovered this ratio ($P \leq 0.05$). H9c2 cells treated with 400 μM PA showed 50% cell viability ($P \leq 0.05$), all other concentrations of the compounds had cell viability > 60% ($P \leq 0.05$), including H9c2 cells treated with 150 μM PA, 15 mM glucosa, and 1 mM PFD ($P \leq 0.05$). H9c2 cells treated with 150 μM and 200 μM PA showed a significant increase in intracellular lipid accumulation ($P \leq 0.001$), and H9c2 cells treated with 150 μM PA and 1.5 mM PDF showed a tendency to reduce intracellular lipid levels.

Conclusions: PFD restores the expression levels of metabolic hormones, which are involved in lipids and carbohydrates metabolism, improving lipid and aminotransferases levels, thus preventing myocarditis and fibrosis in MASH mice. These findings suggest the potential of PFD for the prevention of myocarditis and fibrosis in obesity-induced MASH mice.

Ethical statement: CUCS Research Committee at the University of Guadalajara approved this study (protocol number: CI-01419, CI-02423).

Declaration of interests: None.

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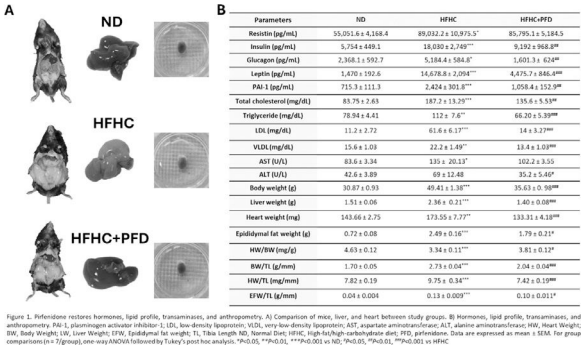

Parameters	ND	HFHC	HFHC+PFD
Resistin (pg/mL)	55,051.6 ± 4,168.4	89,032.2 ± 10,975.5*	85,795.1 ± 5,184.5
Insulin (pg/mL)	5,754 ± 449.1	18,030 ± 2,749****	9,192 ± 968.8##
Glucagon (pg/mL)	2,368.1 ± 592.7	5,184.4 ± 584.8*	1,601.3 ± 624##
Leptin (pg/mL)	1,470 ± 192.6	14,678.8 ± 2,094****	4,475.7 ± 846.4###
PAI-1 (pg/mL)	715.3 ± 111.3	2,424 ± 301.8****	1,058.4 ± 152.9##
Total cholesterol (mg/dL)	83.75 ± 2.63	187.2 ± 13.29****	135.6 ± 5.53##
Triglyceride (mg/dL)	78.94 ± 4.41	112 ± 7.6**	66.20 ± 5.39###
LDL (mg/dL)	11.2 ± 2.72	61.6 ± 6.17****	14 ± 3.27###
VLDL (mg/dL)	15.6 ± 1.03	22.2 ± 1.49**	13.4 ± 1.03###
AST (U/L)	83.6 ± 3.34	135 ± 20.13*	102.2 ± 3.55
ALT (U/L)	42.6 ± 3.89	69 ± 12.48	35.2 ± 5.46#
Body weight (g)	30.87 ± 0.93	49.41 ± 1.38****	35.63 ± 0.98###
Liver weight (g)	1.51 ± 0.06	2.36 ± 0.21****	1.40 ± 0.08###
Heart weight (mg)	143.66 ± 2.75	173.55 ± 7.77****	133.31 ± 4.18###
Epididymal fat weight (g)	0.72 ± 0.08	2.49 ± 0.16****	1.79 ± 0.21#
HW/BW (mg/g)	4.63 ± 0.12	3.34 ± 0.11****	3.81 ± 0.12#
BW/TL (g/mm)	1.70 ± 0.05	2.73 ± 0.04****	2.04 ± 0.04###
HW/TL (mg/mm)	7.82 ± 0.19	9.75 ± 0.34****	7.42 ± 0.19###
EFW/TL (g/mm)	0.04 ± 0.004	0.13 ± 0.009****	0.10 ± 0.011#

Figure 1. Pirfenidone restores hormones, lipid profile, transaminases, and anthropometry. A) Comparison of mice, liver, and heart between study groups. B) Hormones, lipid profile, transaminases, and anthropometry. PAI-1, plasminogen activator inhibitor-1; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HW, Heart Weight; BW, Body Weight; LW, Liver Weight; EFW, Epididymal fat weight; TL, Tiba Length ND, Normal Diet; HFHC, high-fat/high-carbohydrate diet; PFD, pirfenidone. Data are expressed as mean ± SEM. For group comparisons (n = 7/group), one-way ANOVA followed by Tukey's post hoc analysis. *P<0.05, **P<0.01, ***P<0.001 vs ND; #P<0.05, ##P<0.01, ###P<0.001 vs HFHC

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Large volume paracentesis: Is there a limit?

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Introduction and Objectives: Ascites is observed in 5-10% of cirrhotic patients. Large volume paracentesis (LVP), where >5 liters are drained, is safe. Albumin is essential to prevent post-paracentesis circulatory dysfunction (PPCD), with the literature indicating that its incidence increases when draining >8 liters in one session, suggesting draining a smaller amount.

Materials and Patients: An observational, analytical, and retrospective study was conducted, which included the clinical records of patients over 18 years of age admitted to the Gastroenterology service of the General Hospital of Mexico "Dr. Eduardo Liceaga" from January 2020 to March 2024 with a diagnosis of Grade II or III ascites, without criteria for acute kidney injury (AKI) according to the International Ascites Club (ICA) and with baseline creatinine available in