Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Pegbelfermin (BMS-986036), PEGylated FGF21, in Patients with Obesity and Type 2 Diabetes: Results from a Randomized Phase 2 Study

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Objective: Obesity and type 2 diabetes mellitus (T2DM) are risk factors for nonalcoholic fatty liver disease, including nonalcoholic steatohepatitis. This study assessed pegbelfermin (BMS-986036), recombinant PEGylated human fibroblast growth factor 21 (FGF21), in patients with obesity and T2DM predisposed to fatty liver.

Methods: In this randomized, double-blind, placebo-controlled study, patients with T2DM and BMI of 30 to 50 kg/m^2 received subcutaneous pegbelfermin (1, 5, or 20 mg daily or 20 mg weekly; $n=96$) or placebo ($n=24$) for 12 weeks. Primary end points were safety, tolerability, and change in HbA1c. Additional end points included insulin sensitivity, lipids, adiponectin, and disease progression biomarkers.

Results: There were no significant effects of pegbelfermin versus placebo on HbA1c. Pegbelfermin 20 mg/d significantly improved high-density lipoprotein cholesterol ($P=0.015$) and triglycerides ($P=0.037$). All pegbelfermin regimens significantly increased adiponectin levels; 20-mg daily and weekly regimens decreased serum PRO-C3. Most adverse events were mild; the most frequent adverse events were injection-site bruising and diarrhea.

Conclusions: Twelve-week pegbelfermin treatment did not impact HbA1c concentrations, but QW and higher daily doses were associated with improved metabolic parameters and fibrosis biomarkers in patients with obesity and T2DM predisposed to fatty liver. These results support evaluation of pegbelfermin in patients with obesity-related metabolic diseases (e.g., nonalcoholic steatohepatitis).

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Introduction

Approximately 60% of patients with type 2 diabetes mellitus (T2DM) are estimated to have obesity (1,2). Additionally, >50% of patients with T2DM are estimated to have nonalcoholic fatty liver disease (NAFLD) (3), defined as excess accumulation of fat in the liver in the absence of secondary causes for hepatic fat accumulation. Nonalcoholic steatohepatitis (NASH), the most severe form of NAFLD, may progress to cirrhosis and hepatocellular carcinoma (4).

Fibroblast growth factor 21 (FGF21), a key regulator of energy metabolism, is upregulated in NASH (5). Endogenous FGF21 has a short half-life; analogs with extended half-lives improve insulin sensitivity and lipid profiles in preclinical and clinical studies (5-7). Pegbelfermin (BMS-986036) is a polyethylene glycol-modified (PEGylated) recombinant human FGF21 analog with a prolonged half-life that supports up to weekly dosing (Bristol-Myers Squibb [BMS] data on file). In animal models, pegbelfermin has been shown to improve the histologic features of NASH and fibrosis (8), increase levels of adiponectin (a key adipokine with insulin-sensitizing, anti-inflammatory, and antifibrotic properties) (9), and decrease the serum levels of N-terminal type III collagen propeptide (PRO-C3) (BMS data on file), an emerging biomarker of liver fibrosis (10,11). A phase 2 study was conducted to assess the effects of 12-week treatment with daily or weekly administration of pegbelfermin in patients with obesity and T2DM, a population at risk for developing NASH.

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Methods

Inclusion and exclusion criteria

Adults 21 to 75 years old with BMI of 30 to 50 kg/m^2, a diagnosis of T2DM (12,13), a glycated hemoglobin (HbA1c) level ≥ 6.5% and < 10%, and a fasting C-peptide level > 1.0 ng/mL, who were on a stable diet and exercise routine with or without metformin for ≥ 12 weeks prior to the first day of study drug dosing, were enrolled. Patients with fasting plasma glucose > 240 mg/dL, ≥ 5% weight loss within 8 weeks of screening, symptoms of poorly controlled diabetes or a history of any antihyperglycemic therapy other than metformin for > 3 consecutive days or 7 nonconsecutive days within 12 weeks of screening, a history of insulin therapy within 1 year of screening, osteoporosis, or any history of diabetic ketoacidosis, hyperosmolar nonketotic coma, or bariatric surgery were excluded. Patients with recent alcohol abuse (within 6 months of enrollment) as defined by *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) criteria were excluded as were patients who tested positive for hepatitis C antibody or hepatitis B surface antigen.

Study design and procedures

In this phase 2, randomized, double-blind, placebo-controlled, parallel-group study (MB130-002, NCT02097277), patients who completed screening were enrolled in a lead-in period and received training in subcutaneous injection, finger-stick procedures, glucose monitoring, and diary completion. Patients who completed the lead-in phase and were ≥ 80% adherent to treatment were randomized (1:1:1:1:1) to receive placebo, pegbelfermin 1 mg daily, 5 mg daily, 20 mg daily, or 20 mg weekly, all subcutaneously administered. Randomization occurred according to a computer-generated scheme. After the 12-week treatment period, patients were monitored for an additional 6 weeks. All patients were eligible for open-label rescue medication for hyperglycemia.

Study objectives

The primary objective was to assess safety and tolerability of pegbelfermin and its effects on HbA1c levels in patients with T2DM. Secondary objectives included assessing the effects of pegbelfermin on body weight, waist circumference, BMI, glucose homeostasis, and insulin sensitivity (fasting and following oral glucose tolerance test [OGTT]). Exploratory objectives included assessing effects of pegbelfermin on plasma lipids, total adiponectin, serum PRO-C3, chitinase-3-like protein 1 (CHI3L1, also known as YKL-40), a biomarker of liver fibrosis (14), and plasminogen activation inhibitor-1 (PAI-1), a NASH biomarker (15).

Study assessments

Adverse events (AEs) were monitored throughout the study. Hematology parameters, serum chemistry, and urinalysis were evaluated at screening, on days 1, 15, 29, 57, and 84 of the treatment period, at study discharge (day 126), and at all follow-up visits. Body weight was measured at all study visits. HbA1c was measured at screening, on days 1, 29, 43, 57, and 84 of the treatment period, and at study discharge. Fasting plasma glucose and insulin levels (LabCorp Clinical Trials, Cranford, New Jersey) were measured prior to dosing with study medication on days 8, 15, 29, 57, 91, and 105 and prior to a standard 75-g OGTT on days 1, 43, and 84. For the OGTT, blood samples were collected at 0, 30, 60, and 120 minutes post glucose ingestion. Biomarkers of glucose homeostasis were evaluated in the fasting state and after OGTT to calculate insulin sensitivity indices, including the Matsuda index, the homeostatic model assessment of insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI). Fasting serum total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein (HDL) cholesterol (LabCorp) were measured at days 1, 43, 84, and 105. Fasting serum triglycerides (LabCorp) and adiponectin (Myriad RBM, Austin, Texas) were measured on days 1, 8, 15, 29, 43, 57, 84, 91, and 105. Serum PRO-C3 was measured by enzyme-linked immunosorbent assay (ELISA) (Nordic Bioscience, Herlev, Denmark) on days 1, 43, and 84. Plasma PAI-1 and YKL-40 levels were measured by microsphere-based, multiplex immunoassays (Myriad RBM) on days 1, 43, 57, and 84. Dual-energy x-ray absorptiometry was performed to monitor bone mineral density and body composition at screening, end of treatment, and 6 months after the end of treatment.

Statistical methods

A planned randomization of 120 patients (24/group) was estimated to provide ≥ 88% power to detect a difference of 0.7% in HbA1c mean change from baseline to week 12 at a one-sided significance level of 0.05. Changes from baseline to week 12 in HbA1c, body weight, HOMA-IR, QUICKI, Matsuda index, glucose area under the curve, OGTT insulin, and C-peptide were analyzed using a longitudinal repeated-measures analysis model, which provided least-squares mean estimates, standard error of the mean (SE), and two-sided 90% confidence intervals for within-group changes from baseline and between-group differences at all time points. Post hoc longitudinal repeated-measures analyses of changes from baseline to week 12 were performed for adiponectin, low-density lipoprotein, HDL, and triglycerides. PRO-C3, YKL-40, and PAI-1 data were summarized with descriptive statistics. Safety and tolerability parameters, including changes from baseline in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, were summarized by treatment groups. As this was a phase 2 study, there was no plan to adjust alpha for multiplicity because of multiple end points.

Study oversight

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent prior to enrollment. The study protocol, amendments, and informed consent received approval by the Institutional Review Board or Independent Ethics Committee from each study center prior to study initiation. All authors had access to the study data and reviewed and approved the final manuscript.

Results

The study was conducted at 15 sites in the United States and Canada from July 2014 to September 2015. In total, 219 patients were enrolled, 120 were randomized, and 108 (90%) completed double-blind treatment (Figure 1).

Patient demographics and baseline disease characteristics were generally similar across groups (Table 1). Most patients were white (79%), and the mean BMI was 35 kg/m^2. Nearly all patients (97%) had a fatty liver index ≥ 60, consistent with NAFLD (16); 21% had an NAFLD fibrosis score > 0.68, and 4% had a Fibrosis-4 score > 2.67, which were

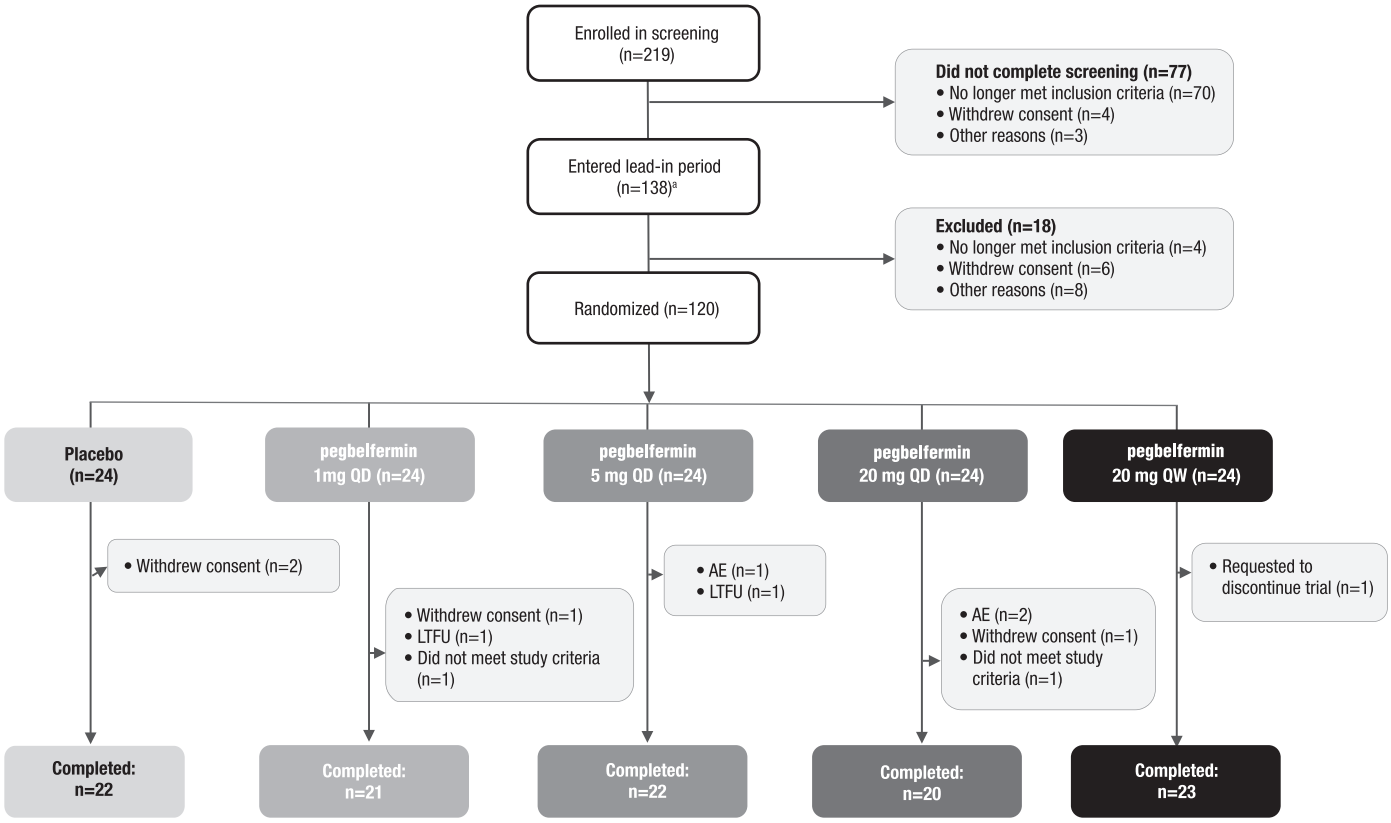

Figure 1 CONSORT flow diagram. [a]Four individuals completed screening but did not receive at least one dose of placebo and were not considered to have entered the lead-in period. AE, adverse event; LTFU, lost to follow-up; QD, daily dosing; QW, weekly dosing.

suggestive of advanced fibrosis (17,18). Although most patients' ALT and AST levels were within the laboratory-defined normal limit, 48% (57/120) were above an updated upper limit of normal (ULN) that has shown a higher sensitivity in identifying patients with liver disease (19). Sixty-eight (57%) patients were receiving stable doses of metformin, and fifty-three (44%) were receiving statins.

Metabolic and lipid parameters

There were no statistically significant differences between pegbelfermin treatment groups and placebo in the change in HbA1c levels from baseline to week 12 (Table 2). Additionally, there were no significant differences in body weight, fasting plasma insulin, C-peptide, and measures of hepatic insulin sensitivity (HOMA-IR and QUICKI). Significant differences ($P<0.05$) versus placebo were observed for a decrease in fasting plasma glucose (20 mg weekly) and an increase in whole-body insulin sensitivity (Matsuda index; 20 mg daily). At week 12, significant improvements were observed in HDL and triglycerides for the 20-mg/d regimen versus placebo (Figure 2).

Parameters associated with NASH and liver fibrosis

At 12 weeks, significant increases in adiponectin were observed in all pegbelfermin dosing groups, compared with placebo, with an apparent dose-response relationship (Figure 3a). A post hoc analysis suggested that patients treated with the 20-mg daily and weekly regimens had significantly decreased PRO-C3 levels compared with placebo-treated patients (Figure 3b). Exploratory analyses also suggested pegbelfermin-associated decreases in PAI-1 (Figure 3c) and YKL-40 (Figure 3d); statistical inference testing was not performed for PAI-1 or YKL-40.

ALT and AST

ALT and AST levels decreased in patients with baseline elevations of those enzymes (ALT > 44 U/L for men or > 32 U/L for women, 20%; AST > 40 U/L, 8%; Table 1). There were no clear differences in decreases observed between pegbelfermin and placebo groups (Figure 4). Overall, there were no apparent on-treatment changes in ALT or AST levels, regardless of the enzyme values at baseline (data not shown).

Safety and tolerability

The most frequently reported treatment-related AEs overall were injection site bruising (5%) and injection site reactions (4%); rates of these AEs were similar between placebo and pegbelfermin treatment groups. Most AEs were mild and did not appear to be dose dependent. In all, 54% of placebo-treated patients experienced an AE compared with 67% (48/72) across all daily dosing groups and 54% in the 20

TABLE 1 Baseline demographics and clinical characteristics

		Pegbelfermin				
	Placebo (*n* = 24)	1 mg QD (*n* = 24)	5 mg QD (*n* = 24)	20 mg QD (*n* = 24)	20 mg QW (*n* = 24)	All patients (*n* = 96)
Male, *n* (%)	14 (58)	13 (54)	13 (54)	15 (63)	12 (50)	53 (55)
Age, y, mean ± SD	58 ± 8	55 ± 9	55 ± 10	56 ± 8	55 ± 13	56 ± 10
White, *n* (%)	22 (92)	20 (83)	18 (75)	20 (83)	15 (63)	73 (76)
Body weight, kg, mean ± SD	97 ± 18	95 ± 17	94 ± 13	95 ± 14	103 ± 23	97 ± 17
BMI, kg/m^2, mean ± SD	36 ± 5	35 ± 3	34 ± 3	34 ± 3	36 ± 6	35 ± 4
Concomitant diabetes medication use, *n* (%)						
Glimepiride	1 (4)	0	1 (4)	0	0	2 (2)
Glyburide	0	0	1 (4)	0	0	1 (1)
Insulin	0	0	0	1 (4)	0	1 (1)
Metformin	13 (54)	13 (54)	15 (63)	14 (58)	13 (54)	55 (57)
Statin use, *n* (%)	10 (42)	12 (50)	10 (42)	12 (50)	9 (38)	43 (45)
Fatty liver index, mean (range)[a]	94 (37-100)[b]	88 (63-99)	84 (46-98)	88 (68-99)	89 (45-100)[b]	91 (45-100)[c]
HbA1c, %, mean ± SD	7.6 ± 0.7	7.7 ± 0.8	7.7 ± 0.9	7.5 ± 1.0	7.7 ± 0.7	7.8 ± 1.0
NAFLD score >0.68, *n* (%)	4 (17)	9 (38)	2 (8)	3 (13)	6 (25)	20 (21)
Fasting plasma glucose, mg/dL, mean ± SD	160 ± 33	151 ± 33	147 ± 36	151 ± 30	154 ± 50[b]	151 ± 37
Fasting plasma insulin, pmol/L, mean ± SD	115 ± 65	152 ± 91	180 ± 194	138 ± 88	169 ± 107[d]	160 ± 126
ALT, U/L, mean ± SD	31 ± 25[b]	28 ± 11	31 ± 20[d]	34 ± 23	26 ± 10[d]	30 ± 17[c]
ALT >44 U/L (men)[e] or >32 U/L (women)[e], *n* (%)	4 (17)	5 (21)	4 (17)	8 (33)	3 (13)	20 (21)
ALT >30 U/L (men) or >19 U/L (women), *n* (%)	10 (42)	13 (54)	9 (38)	14 (58)	11 (46)	47 (49)
AST, U/L, mean ± SD	25 ± 17[b]	22 ± 9	23 ± 9[d]	27 ± 15	24 ± 14[d]	24 ± 12[c]
AST >40 U/L, *n* (%)	3 (13)	1 (4)	1 (4)	3 (13)	1 (4)	6 (6)
GGT, U/L, mean ± SD[f]	43 ± 27	48 ± 47	69 ± 123	39 ± 26	55 ± 48	53 ± 71
Body triglycerides, mg/dL, mean ± SD	193 ± 57[b]	166 ± 74	170 ± 108[d]	191 ± 102	211 ± 175[d]	184 ± 117[c]
LDL cholesterol, mg/dL, mean ± SD	102 ± 34[b]	111 ± 29	109 ± 32[d]	89 ± 33	109 ± 34[d]	104 ± 33[c]
HDL cholesterol, mg/dL, mean ± SD	50 ± 19[b]	49 ± 10	48 ± 11[d]	48 ± 12	48 ± 11[d]	48 ±11[c]
Adiponectin, µg/mL, mean ± SD	3.5 ± 1.5[b]	2.7 ± 1.2	3.0 ± 1.2	3.1 ± 1.4	3.0 ± 1.2[d]	2.9 ± 1.2
PRO-C3, µg/mL, mean ± SD[g]	10.9 ± 5.3	11.3 ± 4.4	11.0 ± 6.4	12.1 ± 7.8	10.9 ± 3.5	11.3 ± 5.6

[a]Fatty liver index determined by BMI, waist circumference, triglycerides, and GGT (16).
[b]*n* = 23.
[c]*n* = 92.
[d]*n* = 22.
[e]Laboratory-defined upper limit of normal.
[f]GGT measured in a study sponsor's laboratory.
[g]PRO-C3 data missing for nine subjects at baseline.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; QD, daily dosing; QW, weekly dosing.

mg weekly group. Diarrhea was more frequent in the pegbelfermin groups versus placebo (Table 3); events were mild to moderate in intensity and lasted ≤ 2 days in 64% (9/14) of affected patients. Other more frequently reported AEs in pegbelfermin-treated than in placebo-treated patients included nausea (6% vs. 0%), dyspepsia (5% vs. 0%), and fatigue (4% vs. 0%). Neither of the two serious AEs reported during the treatment period (pegbelfermin, 20 mg/d: mitral and tricuspid valve disease; placebo: basal cell carcinoma of the right ear lobe) were treatment related. The following three patients discontinued treatment because of AEs: a participant in the 20 mg/d group with mitral and tricuspid valve disease, an individual in the 20 mg/d group with a severe injection site reaction (erythema and edema; resolved 49 days after discontinuation), and a participant in the 5 mg/d group with fatigue (resolved 15 days after the last dose). There were no deaths during the study.

Elevated C-reactive protein was the most frequently reported chemistry abnormality, with values > 1.5-fold the ULN in one-third of patients and similar frequency of elevations across dosing groups. Elevations were observed in 31.0% and 31.8% of patients who received pegbelfermin and placebo, respectively, and are unlikely to be clinically meaningful. Values > 2-fold the ULN for ALT and AST were observed in three and two patients, respectively. No participant met predefined criteria for drug-induced liver injury (ALT or AST

TABLE 2 Changes in metabolic parameters at week 12

			Pegbelfermin			
		Placebo (*n* = 24)	1 mg QD (*n* = 24)	5 mg QD (*n* = 24)	20 mg QD (*n* = 24)	20 mg QW (*n* = 24)
HbA1c, %	Change from baseline (90% CI)	−0.03 (−0.29 to 0.23)	0.32 (0.05 to 0.59)	0.19 (−0.07 to 0.46)	0.06 (−0.21 to 0.34)	−0.05 (−0.31 to 0.21)
	P	–	0.940	0.838	0.660	0.463
Body weight, kg	Change from baseline (90% CI)	−0.12 (−1.02 to 0.77)	−0.10 (−1.04 to 0.83)	−0.44 (−1.38 to 0.49)	−1.04 (−1.99 to −0.10)	−0.48 (−1.39 to 0.43)
	P	–	0.512	0.341	0.122	0.323
Plasma glucose AUC(0-2), mmol•h/L	Change from baseline (90% CI)	1.35 (−0.39 to 3.09)	2.41 (0.56 to 4.26)	0.26 (−1.61 to 2.12)	0.08 (−1.77 to 1.94)	−1.62 (−3.46 to 0.22)
	P	–	0.756	0.240	0.206	0.028
Plasma insulin AUC(0-2), pmol•h/L	Change from baseline (90% CI)	−71.3 (−146 to 3.45)	−77.8 (−156 to 0.46)	−61.4 (−142 to 19.4)	−95.8 (−175 to −16.4)	−142 (−222 to −63.0)
	P	–	0.460	0.559	0.354	0.142
C-peptide AUC(0-2), nmol•h/L	Change from baseline (90% CI)	−0.27 (−0.67 to 0.13)	−0.13 (−0.55 to 0.29)	−0.20 (−0.63 to 0.23)	−0.24 (−0.67 to 0.18)	−0.75 (−1.18 to −0.33)
	P	–	0.655	0.578	0.527	0.084
Matsuda index	Change from baseline (90% CI)	−0.25 (−0.86 to 0.37)	−0.34 (−0.99 to 0.32)	−0.23 (−0.89 to 0.43)	0.74 (0.11 to 1.38)	0.47 (−0.17 to 1.11)
	P	–	0.564	0.487	0.033	0.093
HOMA-IR	Change from baseline (90% CI)	−1.38 (−3.11 to 0.35)	0.58 (−1.24 to 2.40)	−0.75 (−2.56 to 1.07)	−2.13 (−3.91 to −0.36)	0.17 (−1.55 to 1.89)
	P	–	0.902	0.662	0.307	0.852
QUICKI	Change from baseline (90% CI)	0.0005 (−0.0034 to 0.0045)	−0.002 (−0.007 to 0.002)	−0.002 (−0.006 to 0.003)	0.005 (0.0004 to 0.009)	0.001 (−0.003 to 0.005)
	P	–	0.795	0.734	0.125	0.461

Changes from baseline, 90% confidence intervals (CIs), and *P* values derived from longitudinal repeated-measures model that included treatment group, study day, and treatment-by-study-day interactions, baseline value, and baseline BMI (categorical variable).
P values not adjusted for multiple testing.
AUC(0-2), area under the curve, 0-2 h; CI, confidence interval; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QD, daily dosing; QUICKI, quantitative insulin sensitivity check index; QW, weekly dosing.

> 3-fold ULN and total bilirubin > 2-fold ULN). Abnormal laboratory elevations in creatine kinase occurred in 7% and 13% of pegbelfermin- and placebo-treated patients, respectively, and were attributed to occupational or recreational physical activity. No patients had a laboratory abnormality of low glucose; however, one individual in the 20 mg/d group experienced asymptomatic clinical hypoglycemia (as judged by the Investigator based on a random finger-stick test) on day 13, attributed to a missed or delayed meal. Rescue medication was required by five patients to treat hyperglycemia (one each in placebo, 1 mg, and 5 mg daily dose groups; two from 20 mg weekly dose group); three patients required medication during the 12-week treatment period, and two patients started medication several days after completing treatment. Most patients (4/5) achieved adequate glucose control with their prescribed medication, while one patient required additional antihyperglycemic medication to achieve adequate glucose control. Mean bone mineral density at any site (the femoral neck, total hip, or lumbar spine) and body composition as measured by dual-energy x-ray absorptiometry were not decreased from baseline in patients who received pegbelfermin compared with placebo at any time point (data not shown).

Discussion

Pegbelfermin was generally safe and well tolerated among patients with obesity and T2DM and a high prevalence of fatty liver. A proportion of these patients may have had undiagnosed NASH, and the rest were probably at risk of developing NASH (16). Furthermore, 4% to 21% were likely to have advanced (stage 3 or 4) fibrosis based upon noninvasive assessments (NAFLD fibrosis and Fibrosis-4 scores). Overall, 12 weeks of pegbelfermin treatment was not associated with a meaningful change in HbA1c or body weight, but it was associated with improvements in metabolic parameters (HDL and triglycerides [20 mg daily]) and fibrosis markers (adiponectin [all dosages] and PRO-C3 [20 mg daily and weekly]).

The absence of a lowering effect on body weight, glucose, and insulin contrasts with observations from preclinical studies in rodents and nonhuman primates (6,20,21). The data presented in this study are consistent with the results of a clinical trial of a different FGF21 analog, in which 4 weeks of treatment with LY2405319 in patients with T2DM did not result in a meaningful change in fasting plasma glucose but did result in significant reductions in fasting plasma insulin (22). The

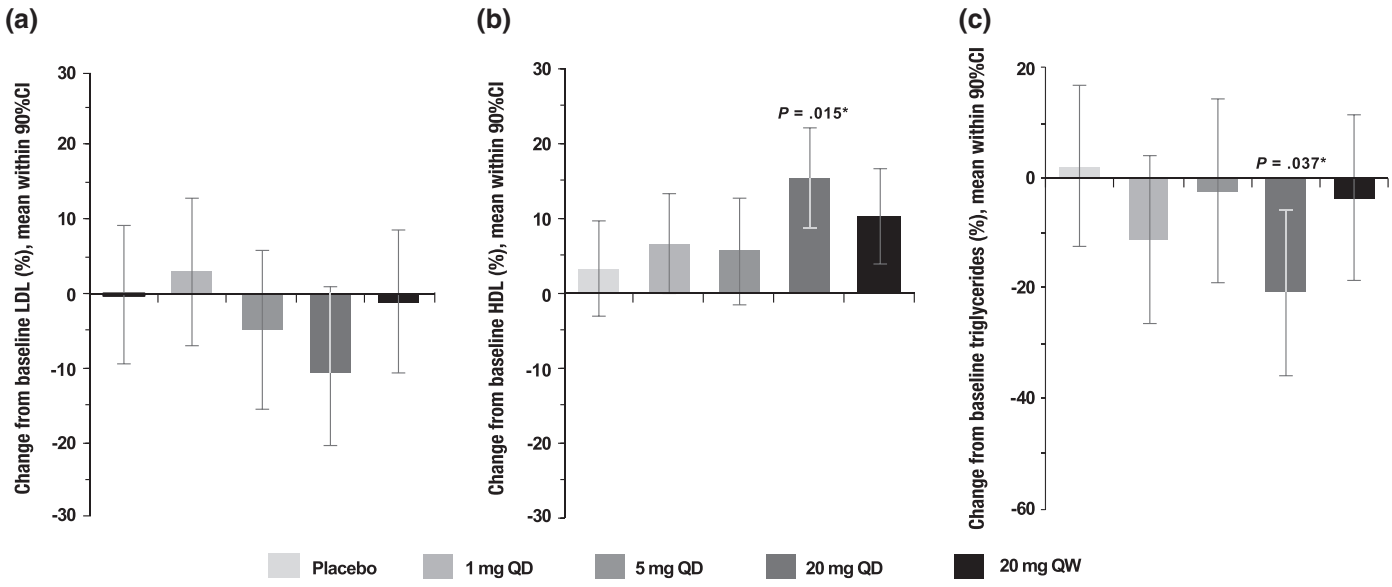

Figure 2 Change from baseline to week 12 in (a) LDL, (b), HDL, and (c) triglycerides. **P* values not adjusted for multiple testing. CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; QD, daily dosing; QW, weekly dosing.

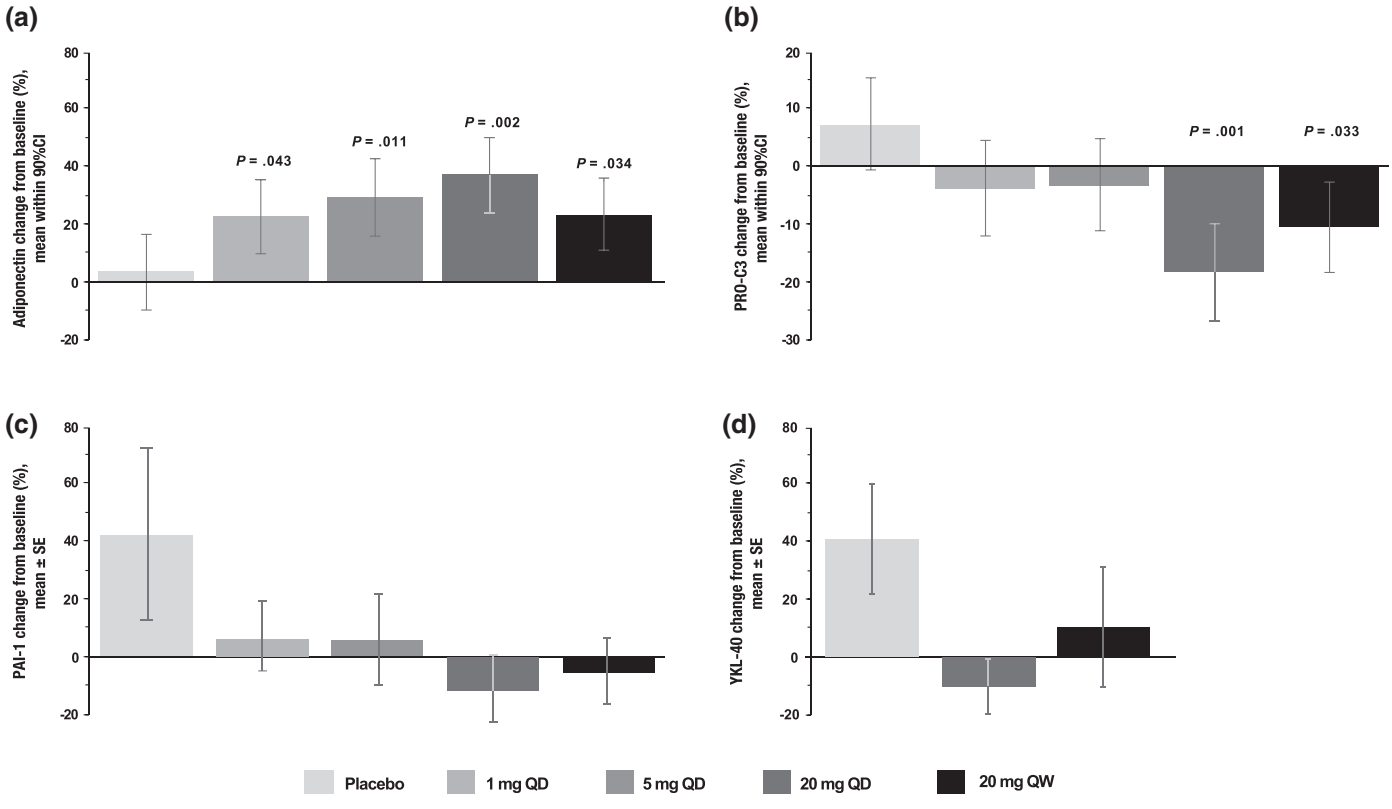

Figure 3 Change from baseline to week 12 in (a) adiponectin, (b) PRO-C3, (c) PAI-1, and (d) YKL-40. CI, confidence interval; PAI-1, plasminogen activator inhibitor-1; PRO-C3, N-terminal type III collagen propeptide; QD, daily dosing; QW, weekly dosing; SE, standard error of the mean; YKL-40, chitinase-3-like protein.

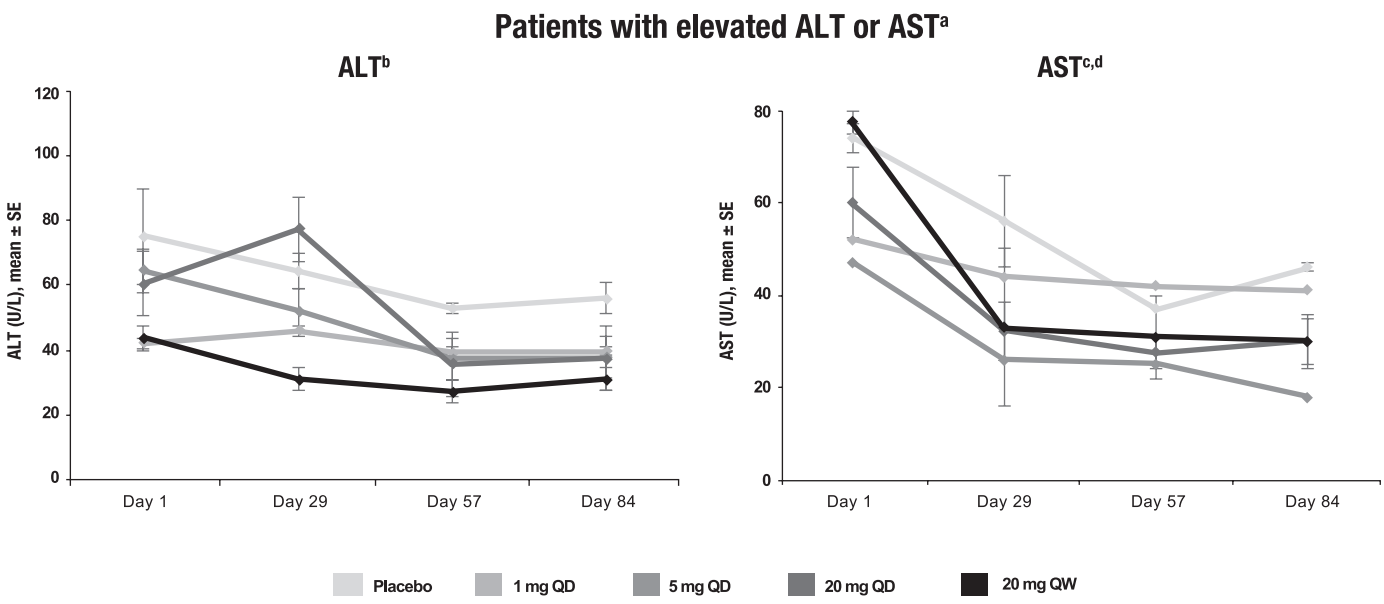

Figure 4 ALT and AST levels during treatment in patients with elevated ALT or AST. [a]ALT >44 U/L (men), >32 U/L (women); AST >40 U/L; [b]$n=24$, [c]$n=10$, and [d]$n=1$ in the 1 mg/d and 5 mg/d groups. ALT, alanine aminotransferase; AST, aspartate aminotransferase; SE, standard error of the mean; QD, daily dosing; QW, weekly dosing.

TABLE 3 Safety summary

Event, *n* (%)	Placebo (*n* = 24)	Pegbelfermin 1 mg QD (*n* = 24)	Pegbelfermin 5 mg QD (*n* = 24)	Pegbelfermin 20 mg QD (*n* = 24)	Pegbelfermin 20 mg QW (*n* = 24)	Pegbelfermin Any dose (*n* = 96)
Any AE	13 (54)	16 (67)	18 (75)	14 (58)	13 (54)	61 (64)
Discontinuation due to AEs	0	0	1 (4)	2 (8)	0	3 (3)
Serious AEs	1 (4)	0	0	1 (4)	0	1 (4)
AEs in >5% of patients in any treatment group						
Diarrhea	1 (4)	3 (13)	2 (8)	4 (17)	5 (21)	14 (15)
Injection site bruising	5 (21)	6 (25)	4 (17)	1 (4)	3 (13)	14 (15)
Nasopharyngitis	1 (4)	3 (13)	1 (4)	1 (4)	2 (8)	7 (7)
Increased appetite	2 (8)	1 (4)	3 (13)	1 (4)	1 (4)	6 (6)
Nausea	0	2 (8)	0	1 (4)	3 (13)	6 (6)
Dyspepsia	0	3 (13)	0	1 (4)	1 (4)	5 (5)
Fatigue	0	1 (4)	1 (4)	0	2 (8)	4 (4)
Injection site erythema	1 (4)	1 (4)	0	2 (8)	1 (4)	4 (4)
Headache	2 (8)	1 (4)	1 (4)	0	1 (4)	3 (3)
Upper respiratory tract infection	0	0	2 (8)	0	1 (4)	3 (3)
Cough	2 (8)	0	1 (4)	0	1 (4)	2 (2)
Increased blood CPK	0	2 (8)	0	0	0	2 (2)
Vomiting	0	2 (8)	0	0	0	2 (2)

AE, adverse event; CPK, creatine phosphokinase; QD, daily dosing; QW, weekly dosing.

reasons for the differences between preclinical and human studies are unclear, though they may include fundamental differences in human versus nonhuman FGF21 physiology.

At the 20-mg/d dose, pegbelfermin treatment led to improvement in the Matsuda index, a calculated measure of insulin sensitivity in the liver and peripheral tissues (23). This finding is consistent with the increased peripheral insulin sensitivity reported in diet-induced obese mice treated with recombinant FGF21 (20); however, it is important to note that these calculations are moderately correlated with data derived from the euglycemic hyperinsulinemic clamp, a gold standard method, and that they may not accurately reflect insulin resistance in patients with diabetes because of ß-cell dysfunction (24). In contrast, there was no observable effect on HOMA-IR and QUICKI, indices that are thought to reflect insulin sensitivity in the liver and pancreatic β-cells (23). It is unclear why the Matsuda index improved at the 20-mg/d dose in the absence of change in HOMA-IR and QUICKI; however, improvement in whole-body insulin sensitivity suggests a possible effect of pegbelfermin treatment on insulin resistance, though additional research is needed to confirm this observation. Insulin resistance is a risk factor for NASH (25), and presence of diabetes is an independent predictor of moderate to severe fibrosis and progression to adverse long-term clinical outcomes in individuals with NAFLD (26,27).

The improvements in HDL and triglycerides observed in the 20 mg/d treatment group are consistent with findings from clinical trials of other FGF21 analogs and studies of exogenous FGF21 in obese rhesus monkeys with dyslipidemia (6,22). These findings, if confirmed in subsequent studies, could be of clinical significance, as a lipid-lowering agent could reduce the overall cardiovascular risk of patients with obesity and T2DM (28).

Compared with placebo, all pegbelfermin treatment groups experienced dose-dependent increases in adiponectin, an adipokine associated with hepatic benefits. Adiponectin levels are decreased in patients with NAFLD and are even lower in patients with NASH (29). In addition, in patients with NAFLD, adiponectin expression decreases with the development of insulin resistance, which may predispose patients to NASH (30). Adiponectin regulates the activation of hepatic stellate cells, major profibrotic protagonists (31), and *in vitro*, adiponectin has a potent antifibrotic effect in fibroblast cultures (32). In animals, recombinant adiponectin alleviates hepatomegaly and steatosis, attenuates inflammation, reduces ALT elevations, and has beneficial effects on lipid metabolism (33). In humans, low adiponectin is correlated with obesity and insulin resistance (34). In a recent meta-analysis of clinical trials with antidiabetes medications pioglitazone and rosiglitazone, increases in adiponectin were associated with improvements in steatosis and fibrosis (35).

The significant effect on serum PRO-C3 in patients treated with 20 mg pegbelfermin daily and weekly is also of clinical interest. PRO-C3 is a biomarker for type III collagen formation (10) and is indicative of hepatic fibrogenesis. For example, in patients with chronic hepatitis C virus infection, PRO-C3 has been shown to correlate with severity of liver fibrosis (10), and high baseline PRO-C3 levels have been associated with increased fibrotic disease progression (36). In patients with NASH, PRO-C3 levels have been associated with disease activity (37) and fibrosis stage (38). Furthermore, longitudinal decreases in PRO-C3 have been correlated with improvements in hepatic fibrosis, as assessed by biopsy (39).

Pegbelfermin treatment also led to reduced levels of PAI-1 and YKL-40, though statistical inference testing was not performed for PAI-1 or YKL-40. PAI-1 is associated with fibrosis (40), and it has been shown to be elevated in patients with NASH (41). YKL-40 is a marker of liver fibrosis (14) and has been associated with fibrosis in NAFLD (42). To our knowledge, no other FGF21 study, in humans or animals, has reported changes in serum PRO-C3, PAI-1, or YKL-40. Future studies will need to confirm the effect of pegbelfermin on these biomarkers and elucidate in more detail their relationship with established markers of hepatic fibrosis.

A key limitation of this trial is the small sample size, which limits interpretation of data. In addition, to more definitively assess the long-term effects of pegbelfermin, studies with a longer treatment duration are needed. Finally, although results from this study suggest that pegbelfermin improves biomarkers relevant to NASH in a patient population with NASH risk factors, whether pegbelfermin improves NASH, as measured by histologic assessment of liver biopsy in NASH patients, will require further study.

In summary, these results suggest that pegbelfermin is generally safe, well tolerated, and associated with improvement in liver-related biomarkers and multiple metabolic parameters in patients with obesity and T2DM who have a high prevalence of fatty liver and who are at risk for developing NASH. These results, together with data from a recent phase 2 study in patients with NASH (A. Sanyal et al., unpublished data, 2018), support larger, long-term studies of pegbelfermin in patients with metabolic syndrome-related disorders and, in particular, NASH.O

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References

1. Preis SR, Pencina MJ, Hwang SJ, et al. Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study. *Circulation* 2009;120:212-220.
2. Selvin E, Parrinello CM, Sacks DB, Coresh J. Trends in prevalence and control of diabetes in the United States, 1988–1994 and 1999–2010. *Ann Intern Med* 2014;160:517-525.
3. Vernon G, Baranova A, Younossi Z. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274-285.
4. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372-384.
5. Kharitonenkov A, Shiyanova TL, Koester A, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest* 2005;115:1627-1635.
6. Kharitonenkov A, Wroblewski VJ, Koester A, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology* 2007;148:774-781.
7. Zhang J, Li Y. Fibroblast growth factor 21 analogs for treating metabolic disorders. *Front Endocrinol (Lausanne)* 2015;6:168.
8. Krupinski J, Morgan N, Kozhich A, Chiney M, Morin P, Christian R. Effects of BMS-986036 (pegylated fibroblast growth factor 21) on hepatic steatosis and fibrosis in a mouse model of nonalcoholic steatohepatitis. *Hepatology* 2016;64:749A.
9. Polyzos SA, Kountouras J, Zavos C, Tsiaousi E. The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. *Diabetes Obes Metab* 2010;12:365-383.

10. Nielsen MJ, Kazankov K, Leeming DJ, et al. Markers of collagen remodeling detect clinically significant fibrosis in chronic hepatitis C patients. *PLoS One* 2015;10:e0137302. doi:10.1371/journal.pone.0137302
11. Nielsen MJ, Veidal SS, Karsdal MA, et al. Plasma Pro-C3 (N-terminal type III collagen propeptide) predicts fibrosis progression in patients with chronic hepatitis C. *Liver Int* 2015;35:429-437.
12. Chamberlain JJ, Rhinehart AS, Shaefer CF Jr, Neuman A. Diagnosis and management of diabetes: synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. *Ann Intern Med* 2016;164:542-552.
13. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee; Booth G, Cheng AY. Canadian Diabetes Association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. Methods. *Can J Diabetes* 2013;37:S4-S7.
14. Huang H, Wu T, Mao J, et al. CHI3L1 is a liver-enriched, noninvasive biomarker that can be used to stage and diagnose substantial hepatic fibrosis. *OMICS* 2015;19:339-345.
15. Ajmera V, Perito ER, Bass NM, et al. Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. *Hepatology* 2017;65:65-77.
16. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;6:33. doi:10.1186/1471-230X-6-33
17. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846-854.
18. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009;7:1104-1112.
19. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002;137:1-10.
20. Xu J, Stanislaus S, Chinookoswong N, et al. Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models-association with liver and adipose tissue effects. *Am J Physiol Endocrinol Metab* 2009;297:E1105-E1114.
21. Coskun T, Bina HA, Schneider MA, et al. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* 2008;149:6018-6027.
22. Gaich G, Chien JY, Fu H, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metab* 2013;18:333-340.
23. Borai A, Livingstone C, Kaddam I, Ferns G. Selection of the appropriate method for the assessment of insulin resistance. *BMC Med Res Methodol* 2011;11:158. doi:10.1186/1471-2288-11-158
24. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Pract Res Clin Endocrinol Metab* 2003;17:305-322.
25. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009;29:113-119.
26. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389-397.e10.
27. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017;14:32-42.
28. Jellinger PS, Handelsman Y, Rosenblit PD, et al. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of atherosclerosis. *Endocr Pract* 2017;23:479-497.
29. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* 2011;60:313-326.
30. Takaki A, Kawai D, Yamamoto K. Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* 2014;15:7352-7379.
31. Shafiei MS, Shetty S, Scherer PE, Rockey DC. Adiponectin regulation of stellate cell activation via PPARgamma-dependent and -independent mechanisms. *Am J Pathol* 2011;178:2690-2699.
32. Fang F, Liu L, Yang Y, et al. The adipokine adiponectin has potent anti-fibrotic effects mediated via adenosine monophosphate-activated protein kinase: novel target for fibrosis therapy. *Arthritis Res Ther* 2012;14:R229. doi:10.1186/ar4070
33. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003;112:91-100.
34. Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 2003;148:293-300.
35. Polyzos SA, Mantzoros CS. Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: a systematic review. *Metabolism* 2016;65:1297-1306.
36. Karsdal MA, Henriksen K, Nielsen MJ, et al. Fibrogenesis assessed by serological type III collagen formation identifies patients with progressive liver fibrosis and responders to a potential antifibrotic therapy. *Am J Physiol Gastrointest Liver Physiol* 2016;311:G1009-G1017.
37. Leeming DJ, Grove JI, Kaye P, et al. Estimation of serum "true collagen type III formation" (Pro-C3) levels as a marker of non-alcoholic steatohepatitis in a prospective cohort. *J Hepatol* 2017;66:S154.
38. Leeming DJ, Nielsen MJ, Goodman Z, et al. Plasma collagen III type III (Pro-C3) levels associate with severity of histological features of non-alcoholic steatohepatitis and fibrosis within the screening population from the CENTAUR study. Presented at: NASH Biomarkers Workshop 2017; May 5, 2017; Washington, DC. http://regist2.virology-education.com/2017/NASHbiomarkers/20_Leeming.pdf.
39. Luo Y, Vincent R, Gagnon RC, et al. Improvement of fibrosis is associated with the decrease of collagen III formation biomarker Pro-C3 in patients with non-alcoholic steatohepatitis [abstract 633]. *Hepatology* 2017;66(suppl 1):345A.
40. Flevaris P, Vaughan D. The role of plasminogen activator inhibitor type-1 in fibrosis. *Semin Thromb Hemost* 2017;43:169-177.
41. Yener S, Akarsu M, Demir T, et al. Plasminogen activator inhibitor-1 and thrombin activatable fibrinolysis inhibitor levels in non-alcoholic steatohepatitis. *J Endocrinol Invest* 2007;30:810-819.
42. Kumagai E, Mano Y, Yoshio S, et al. Serum YKL-40 as a marker of liver fibrosis in patients with non-alcoholic fatty liver disease. *Sci Rep* 2016;6:35282. doi:10.1038/srep35282