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Original Research

Reduction in the risk of major adverse cardiovascular events with the BET protein inhibitor apabetalone in patients with recent acute coronary syndrome, type 2 diabetes, and moderate to high likelihood of non-alcoholic fatty liver disease

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is common among patients with type 2 diabetes mellitus (T2DM) and is associated with increased risk for coronary atherosclerosis and acute cardiovascular (CV) events. We employed the validated, non-invasive Angulo NAFLD fibrosis score (FS) in an intervention study in patients with T2DM and recent acute coronary syndrome (ACS) to determine the association of FS with CV risk and treatment response to apabetalone. Apabetalone is a novel selective inhibitor of the second bromodomain of bromodomain and extra-terminal (BET) proteins, epigenetic regulators of gene expression.

Methods: The Phase 3 BETonMACE trial compared apabetalone with placebo in 2,425 patients with T2DM and recent ACS. In this *post hoc* analysis, we evaluated the impact of apabetalone therapy on CV risk, defined as a composite of major adverse cardiovascular events (MACE: CV death, non-fatal myocardial infarction [MI], or stroke) and hospitalization for heart failure (HHF) in two patient categories of FS that reflect the likelihood of underlying NAFLD. Patients were initially classified into three mutually exclusive categories according to a baseline Angulo FS <-1.455 (F0-F2), -1.455 to 0.675 (indeterminant), and >0.675 (F3-F4), where F0 through F4 connote fibrosis severity none, mild, moderate, severe, and cirrhosis, respectively. The composite of ischemic MACE and HHF in the placebo group was higher in indeterminant and F3-F4 categories compared to the F0-F2 category (17.2% vs 15.0% vs 9.7%). Therefore, for the present analysis, the former two categories were combined into an elevated NAFLD CVD risk group (FS+) that was compared with the F0-F2 group (lower NAFLD risk, $FS_{0\text{-}2}$).

Results: In 73.7% of patients, FS was elevated and consistent with a moderate-to-high likelihood of advanced liver fibrosis (FS+); 26.3% of patients had a lower FS ($FS_{0\text{-}2}$). In the placebo group, FS+ patients had a higher

Abbreviations: ACS, Acute coronary syndrome; ALP, Alkaline Phosphatase; ALT, Alanine aminotransferase; ApoAI, Aproprotein-AI; APR , Acute phase reactant; AST, Aspartate aminotransferase; BD , Bromodoamin; BET, Bromodomain and extraterminal proteinfamily; BMI, Body mass index; CV, Cardiovascular; CVD, Cardiovascular Disease; FS, Fibrosis score; HDL-C, High density lipoprotein cholesterol; HHF, Hospitalization for heart Failure; HR, Hazard ratio; MACE, Major acute coronary event; NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus.

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incidence of ischemic MACE and HHF (15.4%) than FS_{0-2} patients (9.7%). In FS+ patients, addition of apabetalone to standard of care treatment lowered the rate of ischemic MACE compared with placebo (HR = 0.79; 95% CI 0.60-1.05; p=0.10), HHF (HR = 0.53; 95% CI 0.33-0.86; p=0.01), and the composite of ischemic MACE and HHF (HR = 0.76; 95% CI 0.59-0.98; p=0.03). In contrast, there was no apparent benefit of apabetalone in FS_{0-2} patients (HR 1.24; 95% CI 0.75-2.07; p=0.40; HR 1.12; 95% CI 0.30-4.14; p=0.87; and HR 1.13; 95% CI 0.69-1.86; p=0.62, respectively). Over a median duration of 26.5 months, FS increased from baseline in both treatment groups, but the increase was smaller in patients assigned to apabetalone than to placebo (p=0.04).
Conclusions: Amongst patients with T2DM, recent ACS, and a moderate-to-high likelihood of advanced liver fibrosis, apabetalone was associated with a significantly lower rate of ischemic MACE and HHF and attenuated the increase in hepatic FS over time.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is associated with a 2.26-fold higher rate of incident coronary artery disease and a 1.42-fold higher risk of cardiovascular (CV) mortality, but the reasons for the increased cardiovascular disease (CVD) morbidity and mortality are poorly understood as are optimal pharmacological targets for reducing NAFLD associated CVD [1]. Patients with NAFLD have increased risk for coronary artery calcification as well as development of peripheral arterial and cerebrovascular disease [2,3]. NAFLD is highly correlated with multiple CV risk factors, including increased visceral adiposity, insulin resistance, endothelial dysfunction, atherogenic dyslipidemia (increased serum levels of triglyceride-enriched remnant lipoproteins, low high-density lipoprotein (HDL), and elevations in small, dense low-density lipoproteins (LDL)), as well as heightened inflammatory tone and a pro-oxidative and prothrombotic state [4–6]. NAFLD is highly prevalent in patients who are obese or have metabolic syndrome and/or type 2 diabetes mellitus (T2DM) [7,8]. NAFLD is estimated to affect 25-30% of the populations of Western nations and its impact on the risk for CV events tends to be underestimated [9].

Insulin resistance predisposes patients to the development of NAFLD. Progressive hepatic steatosis leads sequentially to increased intrahepatic inflammation, steatosis, fibrosis, and ultimately can result in cirrhosis and end-stage liver disease. It has been shown that inflammation and steatosis in the liver correlate with progression of coronary atherosclerosis and increase risk for acute CV events [10]. Hepatic steatosis is frequently accompanied by other forms of visceral steatosis, such as epicardial fat pad expansion which may act directly and adversely on the coronary arteries and myocardium [11].

The gold standard for diagnosis of NAFLD is liver biopsy. Non-invasive diagnostic techniques include imaging and mechanical testing for liver stiffness (Fibroscan), reflecting fibrosis. However, these techniques are not ordinarily incorporated in the design of large CV outcomes trials. The Angulo fibrosis score (FS) uses 6 readily available variables (age, body mass index [BMI], hyperglycemia/diabetes, aspartate aminotransferase/alanine aminotransferase [AST/ALT] ratio, platelet count, and albumin) to calculate a score using an empiric formula [12]. The score was validated in patients with biopsy-verified NAFLD and shown to predict histologically-determined liver fibrosis severity with high negative and positive predictive value. These findings suggest that the Angulo FS is a useful non-invasive tool to distinguish patients with or without advanced fibrosis [12].

Apabetalone is a novel selective bromodomain (BD) 2-inhibitor of bromodomain and extra-terminal (BET) proteins, epigenetic regulators of gene expression. There is evidence in humans that apabetalone beneficially impacts the lipid profile, endothelial dysfunction, and factors that regulate vascular calcification, inflammation, oxidative tone, and thrombotic status [13–15]. *In vitro*, apabetalone increases hepatocyte Apolipoprotein A-I (ApoA1), while decreasing the expression of genes that populate pro-inflammatory, pro-atherosclerotic, and pro-thrombotic pathways, including alkaline phosphatase (ALP) and acute phase response (APR) proteins [13,16–18]. Strikingly, chimeric mice with humanized livers respond to apabetalone treatment with less expression of human hepatocyte APR genes [18], supporting the possibility that apabetalone would have favorable effects in patients with NAFLD.

The Phase 3 BETonMACE trial compared apabetalone with placebo in 2,425 patients with T2DM and recent acute coronary syndrome (ACS). Treatment with apabetalone resulted in hazard ratios (HR) of 0.82 (p=0.11) for the primary endpoint of ischemic MACE (CV death, non-fatal myocardial infarction [MI], or stroke) and 0.59 (p=0.03) for the secondary endpoint of hospitalization for heart failure (HHF) [19]. In this *post hoc* analysis of BETonMACE, we evaluated the association of the Angulo FS with CVD risk and the effect of apabetalone on the composite outcome of ischemic MACE and HHF.

2. Methods

2.1. Study design

The design and primary CVD results of the phase 3 BETonMACE trial (https://www.clinicaltrials.gov NCT02586155) have been reported [19–24]. The protocol was approved by the responsible institutional review board or ethics committee at each participating site. In brief, eligible participants had an ACS within 7–90 days prior to randomization, a low high-density lipoprotein cholesterol (HDL-C) level, and a diagnosis of type 2 diabetes. Exclusion criteria included any condition which, in the opinion of the investigator, was likely to prevent the subject from complying with the requirements of or completing the study. Qualifying patients who provided written, informed consent were randomized in a 1:1 ratio to receive apabetalone 100 mg orally twice daily or matching placebo, in addition to high-intensity statin therapy with atorvastatin or rosuvastatin and other clinically defined standard of care. The primary outcome was time to the first occurrence of ischemic MACE, defined as a composite of CV death, non-fatal MI, and stroke. The incidence and time to first occurrence of HHF were secondary outcomes.

The analysis population for the previously reported primary results was applied to the current analyses, consisting of all randomized patients who received any amount of study therapy.

2.2. FS measurements

The Angulo FS is calculated as FS = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m^2]) + (1.13*hyperglycemia/diabetes [yes=1, no=0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [$\times 10^9/L$]) – (0.66*albumin [g/dl]) [12]. Height was measured at baseline. Weight was measured at baseline, week 100, and last visit on treatment. For the purpose of calculating BMI, the last observations for height and weight were carried forward until the next scheduled measurements were obtained. Liver function tests including AST, ALT, and ALP were obtained at baseline and every 2 weeks until week 12, then every 4 weeks until week 28, then every 12 weeks until last visit on treatment. Other biochemical measurements including platelet count and albumin were obtained at baseline, week 24, week 52, and every 24 weeks until last visit on treatment. All parameter measurements were performed by a central laboratory (ICON, Farmingdale, New York). FS scores were consequently assigned to windows corresponding to baseline (last score prior to randomization) and 0.5 (13 to <39 weeks), 1 (39 to <65 weeks),

1.5 (65 to <91 weeks), 2 (91 to <117 weeks) and 2.5 years (≥117 weeks) after randomization; if a patient had multiple values within a given post-baseline window, the first score was included in the analyses.

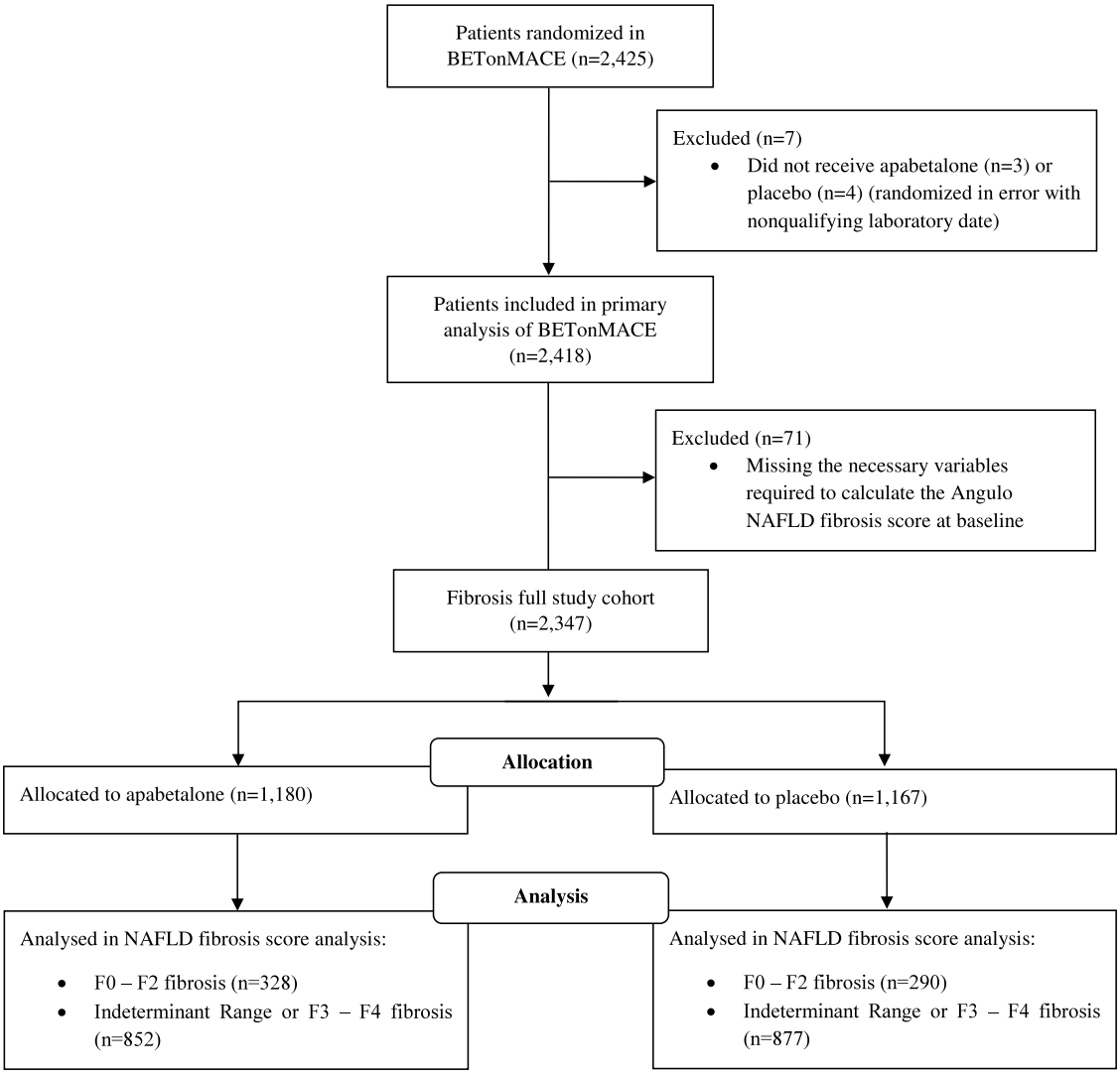
Patients were initially classified into three mutually exclusive categories according to a baseline Angulo FS <-1.455 (F0-F2), -1.455 to 0.675 (indeterminant), and >0.675 (F3-F4), where F0 through F4 connote fibrosis severity none, mild, moderate, severe, and cirrhosis, respectively. The composite of ischemic MACE and HHF in the placebo group was higher in indeterminant and F3-F4 categories compared to the F0-F2 category (17.2% vs 15.0% vs 9.7%). Therefore, for the present analysis, the former two categories were combined into an elevated NAFLD CVD risk group (FS+) that was compared with the F0-F2 group (lower NAFLD risk, $FS_{0\text{-}2}$).

2.3. Statistical analysis

Baseline characteristics were summarized for all patients who had FS data at baseline by FS category (FS+ vs. $FS_{0\text{-}2}$) and further by assigned treatment group. Data were expressed as counts and percentages for categorical variables, mean (standard deviation [SD]) for approximately normal data, or median (interquartile range [IQR]) for non-normal continuous variables. Group comparisons were performed by chi-square tests for categorical variables, z-tests for normal continuous variables, and non-parametric Mann Whitney Wilcoxon tests for non-normal continuous variables.

Change in FS over time was analyzed by a repeated-measures mixed-effects model with absolute change from baseline as the outcome, random effects for intercept and baseline FS score, and fixed effects for treatment group and time, yielding least square (LS) means and corresponding 95% confidence intervals (CIs) for each treatment group and p-values for treatment group differences. Change in FS score was also modeled jointly with time to all-cause death as a sensitivity analysis to account for competing risk. LS means and p-values at each timepoint (0.5, 1, 1.5, 2, and 2.5 years after randomization) were determined by interaction terms between treatment and time. Results were also generated for subgroups defined by baseline FS category.

Possible heterogeneity in relative and absolute effects of apabetalone treatment on ischemic MACE and HHF end points were assessed according to baseline FS category. We constructed a Cox proportional hazards model with risk group, treatment, and their interaction as predictors. Absolute risk reductions (ARRs) were calculated in terms of events per 100 patient-years of follow-up, with a test for quantitative



Patient Flow in the FS subgroups of the BETonMACE trial comparing apabetalone versus placebo.

Fig. 1. Patient Flow in the FS subgroups of the BETonMACE trial comparing apabetalone versus placebo.

interaction between risk group and treatment. Changes in biochemical parameters from baseline to each individual measured time point were analyzed using analysis of covariance models (ANCOVA) with baseline measurements serving as covariates. Results from the ANCOVA analyses are reported as least squares (LS) means with standard error. Analyses were performed with R software, version 3.5.1 or higher (R Foundation for Statistical Computing). P-values less than 0.05 were considered statistically significant without adjustment for multiplicity in this *post-hoc* analysis.

Table 1
Demographics, clinical, pharmacologic, and laboratory characteristics of the BETonMACE trial participants at baseline, according to assigned treatment group, and FS category.

	Full Study Cohort According to Assigned Treatment Group(n = 2,347)			Full Study Cohort According to FS Category(n = 2,347)		
	Placebo	Apabetalone	p-value	FS_{0-2} Patients	FS+ Patients	p-value
Number of Participants	1,167	1,180	–	618	1,729	–
Fibrosis Score	-0.72 (1.29)	-0.81 (1.33)	0.10	**-2.40 (0.97)**	**-0.17 (0.83)**	**<0.0001**
F0 – F2	290 (24.9%)	328 (27.8%)	0.12	618 (100%)	–	–
Indeterminant range	732 (62.7%)	708 (60.0%)	0.19	–	1,440 (83.3%)	–
F3 – F4	145 (12.4%)	144 (12.2%)	0.92	–	289 (16.7%)	–
Demographics						
Age, years	62 (55.5 – 68)	62 (55 – 68)	0.07	**57 (49 – 62)**	**63 (57 – 69)**	**<0.0001**
Female, n (%)	301 (25.8%)	295 (25.0%)	0.69	148 (23.9%)	448 (25.9%)	0.36
White, n (%)	1,018 (87.2%)	1,036 (87.8%)	0.73	**513 (83.0%)**	**1,541 (89.1%)**	**0.0001**
Asian, n (%)	19 (1.6%)	20 (1.7%)	0.97	14 (2.3%)	25 (1.4%)	0.24
Other race, n (%)	130 (11.1%)	124 (10.5%)	0.67	**91 (14.7%)**	**163 (9.4%)**	**0.0004**
Body mass index, kg/m^2	30.3 (5.0)	30.2 (4.8)	0.53	**28.6 (4.2)**	**30.8 (5.0)**	**<0.0001**
Hypertension history, n (%)	1,059 (90.7%)	1,083 (91.8%)	0.42	**510 (82.5%)**	**1,566 (90.6%)**	**<0.0001**
Smoking status, n (%)	125 (10.7%)	147 (12.5%)	0.21	**89 (14.4%)**	**176 (10.2%)**	**0.006**
Diabetes duration, years	8.7 (7.6)	8.4 (7.6)	0.45	**7.3 (6.9)**	**9.0 (7.8)**	**<0.0001**
Blood Pressure, mm Hg						
Systolic	130 (120 – 140)	130 (120 – 140)	0.57	**125 (116 – 135)**	**130 (120 – 140)**	**<0.0001**
Diastolic	77 (70 – 82)	78 (70 – 82)	0.62	76 (70 – 81)	78 (70 – 82)	0.09
Index ACS						
Myocardial infarction, n (%)	865 (74.8%)	862 (73.2%)	0.41	**506 (82.4%)**	**1,221 (71.0%)**	**<0.0001**
NSTEMI, n (%)	403 (46.8%)	401 (46.8%)	0.97	**189 (37.4%)**	**615 (50.7%)**	**<0.0001**
STEMI, n (%)	458 (53.2%)	456 (53.2%)	0.97	**317 (62.6%)**	**597 (49.3%)**	**<0.0001**
Unstable angina, n (%)	291 (25.2%)	315 (26.8%)	0.41	**108 (17.6%)**	**498 (29.0%)**	**<0.0001**
Time from index ACS, days	38 (25 – 62)	38 (25 – 63)	0.48	**31 (23 – 56)**	**40 (26 – 64)**	**<0.0001**
Cardiovascular Medications						
Atorvastatin, n (%)	599 (51.3%)	600 (50.8%)	0.85	299 (48.4%)	900 (52.1%)	0.13
Rosuvastatin, n (%)	568 (48.7%)	580 (49.2%)	0.85	319 (51.6%)	829 (47.9%)	0.13
Intensive statin therapy, n (%)	1,067 (91.4%)	1,067 (90.4%)	0.44	561 (90.8%)	1,573 (91.0%)	0.95
Ezetimibe, n (%)	30 (2.6%)	32 (2.7%)	0.93	18 (2.9%)	44 (2.5%)	0.73
ACE inhibitors or ARB, n (%)	1,073 (91.9%)	1,089 (92.3%)	0.82	**557 (90.1%)**	**1,605 (92.8%)**	**0.04**
Beta-blockers, n (%)	1,052 (90.1%)	1,076 (91.2%)	0.43	549 (88.8%)	1,579 (91.3%)	0.08
Antiplatelet agents, n (%)	1,156 (99.1%)	1,164 (98.6%)	0.46	612 (99.0%)	1,708 (98.8%)	0.79
Diabetes Medications						
Metformin, n (%)	958 (82.1%)	981 (83.1%)	0.54	**530 (85.8%)**	**1,409 (81.5%)**	**0.02**
Insulin, n (%)	446 (38.2%)	437 (37.0%)	0.58	237 (38.3%)	646 (37.4%)	0.70
Sulfonylureas, n (%)	332 (28.4%)	359 (30.4%)	0.32	163 (26.4%)	528 (30.5%)	0.06
DPP4 inhibitors, n (%)	170 (14.6%)	177 (15.0%)	0.81	98 (15.9%)	249 (14.4%)	0.42
SGLT2 inhibitors, n (%)	142 (12.2%)	146 (12.4%)	0.93	82 (13.3%)	206 (11.9%)	0.42
GLP1 receptor agonists, n (%)	43 (3.7%)	40 (3.4%)	0.78	25 (4.0%)	58 (3.4%)	0.50
Biochemical Parameters						
eGFR, mL/min/1.73 m^2	97.3 (75.2 – 125.1)	99.8 (77.3 – 127.1)	0.08	**106.4 (79.5 – 133.9)**	**95.8 (74.5 – 123.3)**	**<0.0001**
HbA1c, %	7.3 (6.4 – 8.6)	7.4 (6.4 – 8.7)	0.36	**7.6 (6.5 – 9.0)**	**7.3 (6.4 – 8.5)**	**0.0001**
Serum glucose, mg/dL	132.9 (109.9 – 174.2)	136.4 (110.6 – 175.3)	0.33	133.1 (109.3 – 172.9)	135.7 (110.8 – 175.5)	0.32
Total cholesterol, mg/dL	129.9 (111.9 – 155.6)	128.4 (109.4 – 155.2)	0.33	129.2 (112.5 – 153.8)	129.5 (109.8 – 157.0)	0.83
LDL cholesterol, mg/dL	65.0 (48.6 – 85.5)	65.0 (49.1 – 85.1)	0.89	65.0 (50.3 – 82.0)	65.2 (48.3 – 86.2)	0.97
HDL cholesterol, mg/dL	33.6 (30.2 – 37.1)	33.3 (29.8 – 37.1)	0.78	**32.9 (29.8 – 36.7)**	**33.6 (30.2 – 37.1)**	**0.008**
Triglycerides, mg/dL	150.6 (115.1 – 201.9)	147.0 (111.6 – 198.4)	0.14	150.1 (113.6 – 205.5)	147.5 (112.5 – 198.4)	0.25
Alkaline phosphatase, U/L	77.0 (64.0 – 93.0)	78.0 (64.0 – 95.0)	0.38	**82.0 (68.0 – 101.8)**	**76.0 (63.0 – 92.0)**	**<0.0001**
Alanine aminotransferase, U/L	22.0 (17.0 – 30.0)	22.0 (17.0 – 31.0)	0.58	**25.0 (18.0 – 34.0)**	**21.0 (16.0 – 29.0)**	**<0.0001**
Aspartate aminotransferase, U/L	19.0 (15.0 – 23.0)	19.0 (15.0 – 23.0)	0.61	18.0 (15.0 – 22.8)	19.0 (15.0 – 23.0)	0.07
AST / ALT, ratio	0.82 (0.69 – 1.00)	0.82 (0.69 – 1.00)	0.73	**0.73 (0.62 – 0.88)**	**0.86 (0.71 – 1.06)**	**<0.0001**
Albumin, g/L	43.0 (41.0 – 45.0)	43.0 (41.0 – 45.0)	0.58	**43.0 (41.0 – 45.0)**	**43.0 (41.0 – 44.0)**	**<0.0001**
Platelets, $10^9/L$	246.0 (206.0 – 296.5)	252.0 (209.0 – 306.0)	0.10	**337.5 (295.0 – 385.0)**	**228.0 (194.0 – 262.0)**	**<0.0001**
Total bilirubin, umol/L	9.2 (6.9 – 12.1)	9.1 (6.8 – 11.9)	0.53	**8.3 (6.4 – 11.0)**	**9.4 (7.1 – 12.4)**	**<0.0001**
hsCRP, mg/L	2.7 (1.1 – 6.1) [n = 238]	3.0 (1.3 – 6.2) [n = 237]	0.52	3.7 (1.3 – 7.0) [n = 124]	2.7 (1.2 – 5.7) [n = 351]	0.16
NLR, ratio	2.6 (2.0 – 3.3)	2.5 (2.0 – 3.3)	0.09	2.6 (2.0 – 3.3)	2.5 (2.0 – 3.4)	0.21

Abbreviations: FS, fibrosis score; FS_{0-2}, F0 – F2 fibrosis; FS+, indeterminant range or F3 – F4 fibrosis; NSTEMI, non-ST segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; ACS, acute coronary syndrome; ACE, angiotensin-converting enzyme; ARB, Angiotensin II receptor blocker; DPP4, dipeptidyl peptidase 4; SGLT2, sodium-glucose cotransporter 2; GLP1, glucagon-like peptide 1; HbA1c, hemoglobin A_{1C}; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hsCRP, high-sensitivity C-reactive protein; NLR, neutrophil-lymphocyte ratio. Categorical variables are presented as n (%). Continuous variables are presented as mean (SD) for normal data or median (quartile 1–quartile 3) for non-normal data. P-values comparing groups at baseline were calculated using chi-square test for categorical variables, z-test for normal continuous variables, and Mann-Whitney Wilcoxon test for non-normal continuous variables. P-values of <0.05 are considered statistically significant and are highlighted in bold.

3. Results

3.1. Demographic features

The BETonMACE trial randomized 2,425 patients to either apabetalone or placebo at 190 sites in 13 countries between November 2015 and July 2018. As shown in Fig. 1, after excluding 7 participants who were randomized in error and an additional 71 participants for whom FS could not be calculated, 2,347 participants were included in this analysis. These participants received at minimum one dose of study drug and were followed for a median of 26.5 months. The analysis cohort included 1,729 (73.7%) patients categorized as FS+ with a mean (SD) FS -0.17

Table 2
Demographics, clinical, pharmacologic, and laboratory characteristics of the BETonMACE trial participants at baseline, according to FS category and assigned treatment group.

	FS_{0-2} Patients According to Assigned Treatment Group (n = 618)			FS+ Patients According to Assigned Treatment Group (n = 1,729)		
	Placebo	Apabetalone	p-value	Placebo	Apabetalone	p-value
Number of Participants	290	328	–	877	852	–
Fibrosis Score	-2.39 (0.98)	-2.42 (0.96)	0.72	-0.16 (0.82)	-0.19 (0.84)	0.57
F0 – F2	290 (100%)	328 (100%)	–	–	–	–
Indeterminant range	–	–	–	732 (83.5%)	708 (83.1%)	–
F3 – F4	–	–	–	145 (16.5%)	144 (16.9%)	–
Demographics						
Age, years	56 (50 – 62)	57 (49 – 62)	0.97	64 (58 – 70)	63 (57 – 69)	0.12
Female, n (%)	69 (23.8%)	79 (24.1%)	0.99	232 (26.5%)	216 (25.4%)	0.64
White, n (%)	**231 (79.7%)**	**282 (86.0%)**	**0.048**	787 (89.7%)	754 (88.5%)	0.45
Asian, n (%)	9 (3.1%)	5 (1.5%)	0.30	10 (1.1%)	15 (1.8%)	0.38
Other race, n (%)	50 (17.2%)	41 (12.5%)	0.12	80 (9.1%)	83 (9.7%)	0.72
Body mass index, kg/m^2	28.5 (4.3)	28.7 (4.1)	0.65	30.9 (5.1)	30.7 (4.9)	0.57
Hypertension history, n (%)	238 (82.1%)	272 (82.9%)	0.86	782 (89.2%)	784 (92.0%)	0.052
Smoking status, n (%)	36 (12.4%)	53 (16.2%)	0.23	84 (9.6%)	92 (10.8%)	0.45
Diabetes duration, years	7.3 (7.1)	7.4 (6.7)	0.90	9.1 (7.8)	8.8 (7.9)	0.45
Blood Pressure, mm Hg						
Systolic	125 (116 – 136)	124 (115 – 134)	0.28	130 (120 – 140)	130 (120 – 140)	0.70
Diastolic	75 (70 – 80)	77 (70 – 81)	0.43	78 (70 – 82)	78 (70 – 82)	0.89
Index ACS						
Myocardial infarction, n (%)	242 (84.6%)	264 (80.5%)	0.22	623 (71.6%)	598 (70.4%)	0.63
NSTEMI, n (%)	92 (38.0%)	97 (36.7%)	0.84	311 (50.2%)	304 (51.3%)	0.77
STEMI, n (%)	150 (62.0%)	167 (63.3%)	0.84	308 (49.8%)	289 (48.7%)	0.77
Unstable angina, n (%)	44 (15.4%)	64 (19.5%)	0.22	247 (28.4%)	251 (29.6%)	0.63
Time from index ACS, days	32 (24 – 56)	31 (23 – 56)	0.64	41 (27 – 63)	40 (26 – 65)	0.67
Cardiovascular Medications						
Atorvastatin, n (%)	146 (50.3%)	153 (46.6%)	0.40	453 (51.7%)	447 (52.5%)	0.77
Rosuvastatin, n (%)	144 (49.7%)	175 (53.4%)	0.40	424 (48.3%)	405 (47.5%)	0.77
Intensive statin therapy, n (%)	265 (91.4%)	296 (90.2%)	0.73	802 (91.4%)	771 (90.5%)	0.54
Ezetimibe, n (%)	6 (2.1%)	12 (3.7%)	0.35	24 (2.7%)	20 (2.3%)	0.72
ACE inhibitors or ARB, n (%)	261 (90.0%)	296 (90.2%)	0.97	812 (92.6%)	793 (93.1%)	0.76
Beta-blockers, n (%)	**248 (85.5%)**	**301 (91.8%)**	**0.02**	804 (91.7%)	775 (91.0%)	0.66
Antiplatelet agents, n (%)	288 (99.3%)	324 (98.8%)	0.80	868 (99.0%)	840 (98.6%)	0.61
Diabetes Medications						
Metformin, n (%)	243 (83.8%)	287 (87.5%)	0.23	715 (81.5%)	694 (81.5%)	0.98
Insulin, n (%)	107 (36.9%)	130 (39.6%)	0.54	339 (38.7%)	307 (36.0%)	0.28
Sulfonylureas, n (%)	77 (26.6%)	86 (26.2%)	1.00	255 (29.1%)	273 (32.0%)	0.20
DPP4 inhibitors, n (%)	50 (17.2%)	48 (14.6%)	0.44	120 (13.7%)	129 (15.1%)	0.43
SGLT2 inhibitors, n (%)	40 (13.8%)	42 (12.8%)	0.81	102 (11.6%)	104 (12.2%)	0.77
GLP1 receptor agonists, n (%)	10 (3.4%)	15 (4.6%)	0.61	33 (3.8%)	25 (2.9%)	0.41
Biochemical Parameters						
eGFR, mL/min/1.73 m^2	105.2 (78.7 – 135.7)	107.6 (80.3 – 132.5)	0.80	94.6 (72.5 – 122.1)	97.1 (75.8 – 125.1)	0.09
HbA1c, %	7.6 (6.5 – 9.0)	7.7 (6.5 – 9.1)	0.77	7.2 (6.4 – 8.5)	7.3 (6.4 – 8.5)	0.49
Serum glucose, mg/dL	131.4 (110.6 – 177.5)	134.1 (108.3 – 168.9)	0.92	133.5 (109.9 – 173.5)	136.9 (111.5 – 176.9)	0.20
Total cholesterol, mg/dL	127.8 (114.2 – 152.8)	130.9 (110.1 – 154.3)	0.75	130.7 (110.6 – 157.8)	127.4 (109.4 – 155.5)	0.20
LDL cholesterol, mg/dL	63.8 (50.0 – 78.9)	66.1 (50.7 – 84.1)	0.31	65.4 (48.0 – 86.5)	65.0 (48.3 – 86.2)	0.47
HDL cholesterol, mg/dL	32.5 (29.4 – 37.0)	32.9 (29.8 – 36.3)	0.76	33.6 (30.2 – 37.1)	33.6 (29.8 – 37.1)	0.71
Triglycerides, mg/dL	149.7 (119.6 – 206.6)	151.0 (111.4 – 205.5)	0.68	150.6 (114.3 – 200.4)	145.3 (111.6 – 195.7)	0.13
Alkaline phosphatase, U/L	82.5 (69.0 – 102.0)	81.0 (67.8 – 101.0)	0.55	76.0 (62.0 – 91.0)	77.0 (63.0 – 93.0)	0.22
Alanine aminotransferase, U/L	25.0 (18.0 – 34.0)	25.0 (18.0 – 34.0)	0.95	22.0 (16.0 – 29.0)	21.0 (16.0 – 29.0)	0.32
Aspartate aminotransferase, U/L	18.0 (15.0 – 22.0)	19.0 (15.0 – 23.0)	0.41	19.0 (15.0 – 23.0)	19.0 (15.0 – 23.0)	0.88
AST / ALT, ratio	0.73 (0.62 – 0.88)	0.73 (0.62 – 0.89)	0.69	0.86 (0.71 – 1.06)	0.87 (0.72 – 1.07)	0.53
Albumin, g/L	43.0 (41.0 – 46.0)	43.0 (41.0 – 45.0)	0.95	43.0 (41.0 – 44.0)	43.0 (41.0 – 44.0)	0.42
Platelets, $10^9/L$	332.0 (293.0 – 384.8)	340.5 (299.8 – 385.0)	0.34	228.0 (189.0 – 262.0)	228.0 (196.0 – 262.0)	0.73
Total bilirubin, umol/L	8.0 (6.4 – 11.0)	8.6 (6.4 – 10.9)	0.38	9.5 (7.1 – 12.4)	9.3 (7.0 – 12.3)	0.34
hsCRP, mg/L	4.0 (1.3 – 7.4) [n = 61]	3.7 (1.3 – 6.8) [n = 63]	0.77	2.3 (1.1 – 5.6) [n = 177]	3.0 (1.3 – 5.7) [n = 174]	0.38
NLR, ratio	**2.7 (2.1 – 3.5)**	**2.4 (1.9 – 3.2)**	**0.001**	2.5 (1.9 – 3.3)	2.6 (2.0 – 3.4)	0.79

Abbreviations: FS, fibrosis score; FS_{0-2}, F0 – F2 fibrosis; FS+, indeterminant range or F3 – F4 fibrosis; NSTEMI, non-ST segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; ACS, acute coronary syndrome; ACE, angiotensin-converting enzyme; ARB, Angiotensin II receptor blocker; DPP4, dipeptidyl peptidase 4; SGLT2, sodium-glucose cotransporter 2; GLP1, glucagon-like peptide 1; HbA1c, hemoglobin A_{1C}; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hsCRP, high-sensitivity C-reactive protein; NLR, neutrophil-lymphocyte ratio. Categorical variables are presented as n (%). Continuous variables are presented as mean (SD) for normal data or median (quartile 1–quartile 3) for non-normal data. P-values comparing groups at baseline were calculated using chi-square test for categorical variables, z-test for normal continuous variables, and Mann-Whitney Wilcoxon test for non-normal continuous variables. P-values of <0.05 are considered statistically significant and are highlighted in bold.

(0.83), and 618 (26.3%) patients categorized as FS_{0-2} with FS -2.40 (0.97). Patient flow through the trial by FS category and treatment assignment is depicted in Fig. 1.

Patients with FS+ scores compared to those with FS_{0-2} scores were older by approximately 6 years, more likely to be White (89.1 vs 83.0%), had higher BMI (30.8 vs 28.6), were more likely to be hypertensive (90.6 vs 82.5%), had higher systolic blood pressure (5 mm Hg between group difference), were less likely to smoke (10.2 vs 14.7%), and had longer duration of T2DM (7.8 vs 6.9 years from diagnosis) (Table 1). There were no clinically meaningful differences between FS categories for cardiovascular or diabetes medication usage. Although there were some statistically significant between-group differences in specific blood chemistries (glycated hemoglobin (HbA1c), HDL-C, ALP, ALT, and total bilirubin), these differences were small and unlikely to be clinically significant. Among FS_{0-2} participants considered by treatment allocation, there were small but significant imbalances in percentage who were White and among those using beta-blockers (Table 2). Among FS+ participants, there were no discernible baseline differences by treatment allocation.

3.2. Impact of apabetalone on cardiovascular outcomes in FS+ and FS_{0-2} participants

In the placebo group, the incidence of ischemic MACE and HHF was greater among patients categorized as FS+ (15.4%) compared to those categorized as FS_{0-2} (9.7%) (Table 3). As shown in Table 3 and Figs. 2–4, the addition of apabetalone to standard of care treatment lowered the rate of ischemic MACE in FS+ patients (HR = 0.79; 95% CI 0.60-1.05; p=0.10) (Fig. 2), HHF (HR = 0.53; 95% CI 0.33-0.86; p=0.01) (Fig. 3), and the composite of ischemic MACE and HHF (HR = 0.76; 95% CI 0.59-0.98; p=0.03) (Fig. 4). In contrast, there was no apparent benefit of apabetalone in FS_{0-2} patients [HR 1.24, 95% CI 0.75-2.07, p=0.40; HR 1.12, 95% CI 0.30-4.14, p=0.87; and HR 1.13, 95% CI 0.69-1.86, p=0.62 for ischemic MACE (Fig. 2), HHF (Fig. 3), and the composite of ischemic MACE and HHF (Fig. 4), respectively]. The interaction p-values of treatment and FS category on risk of ischemic MACE, HHF, and the composite of ischemic MACE and HHF were 0.14, 0.28, and 0.16, respectively (Table 3).

3.3. Changes in FS over time

At baseline, there were no significant differences in FS score between patients assigned to apapbetalone or placebo in either FS category (Table 2). We followed change in FS over time to explore if and how apabetalone influences the score and whether FS associates with the clinical efficacy of apabetalone. Among the patients with a baseline score, 2,142 had at least one post-baseline score and were therefore included in the analyses of change. The LS mean change in FS from baseline to 2.5 years are summarized in Fig. 5A for the full analysis cohort, and in Fig. 5B and 5C for the FS+ and FS_{0-2} categories respectively. At 0.5-years, participants in both treatment groups of both FS categories showed increases in FS that were primarily attributable to increased AST (Supplementary Tables S1-S4). The increase from baseline was smaller in the FS+ category than in the FS_{0-2} category. After the 0.5-year timepoint, FS remained relatively stable in both treatment groups of both FS categories. However, over the course of the trial, considering the full study cohort, the increase in FS from baseline was significantly smaller in patients assigned to apabetalone treatment than in those assigned to placebo (p=0.04, Fig. 5A). This reflected a significantly smaller increase in FS from baseline with apabetalone than with placebo in the FS+ category (p=0.02, Fig. 5B), with no difference between treatment groups in the change in FS from baseline in the FS_{0-2} category (p=0.62, Fig. 5C). The factor that primarily accounted for smaller increases in FS from baseline with apabetalone than placebo in the full analysis cohort and in the FS+ category was a smaller increase in AST/ALT ratio (Supplementary Tables S1-S4).

3.4. Safety

Overall, 68% of the analysis cohort reported at least one adverse event, and 28% reported at least one serious adverse event. There were no discernible imbalances between FS categories or treatment groups (Table 4). Slightly more patients in the FS+ and FS_{0-2} categories discontinued apabetalone than placebo (Table 5). There was a small excess in the number of patients with alanine aminotransferase elevations in the apabetalone group apparent in both FS categories (Table 5). There was no apparent imbalance in aspartate aminotransferase or gamma-glutamyltransferase elevations. Apabetalone therapy did not correlate with increased risk for diabetes, hypertension, diarrhea, or any type of chest pain.

4. Discussion

In this *post hoc* analysis of the BETonMACE trial we calculated the Angulo FS, originally developed as a non-invasive surrogate for histologic liver fibrosis changes in NAFLD, based on the rationale that post-

Table 3
Kaplan-Meier estimates of time to first occurrence of ischemic MACE (CV death, non-fatal MI, or stroke), HHF, and the composite of ischemic MACE and HHF, according to FS category and assigned treatment group. A log-rank test was used for the formal hypothesis testing and a Cox proportional hazards model to estimate the HR with 95% CI. P-values of <0.05 are considered statistically significant.

		FS_{0-2} Patients According to Assigned Treatment Group (n = 618)			FS+ Patients According to Assigned Treatment Group (n = 1,729)			Cox Model Interaction p-valueof HR
		Placebo (n=290)	Apabetalone (n=328)	p-value	Placebo (n=877)	Apabetalone (n=852)	p-value	
Ischemic MACE (CV Death, Non-Fatal MI, or Stroke)	**No. of Events (%)**	25 (8.6%)	34 (10.4%)	–	114 (13.0%)	90 (10.6%)	–	–
	HR (95% CI)	1.24 (0.75 – 2.07)		0.40	0.79 (0.60 – 1.05)		0.10	0.14
HHF	**No. of Events (%)**	4 (1.4%)	5 (1.5%)	–	**43 (4.9%)**	**22 (2.6%)**	–	–
	HR (95% CI)	1.12 (0.30 – 4.14)		0.87	**0.53 (0.33 – 0.86)**		**0.01**	0.28
Composite of Ischemic MACE and HHF	**No. of Events (%)**	28 (9.7%)	35 (10.7%)	–	**135 (15.4%)**	**102 (12.0%)**	–	–
	HR (95% CI)	1.13 (0.69 – 1.86)		0.62	**0.76 (0.59 – 0.98)**		**0.03**	0.16

Abbreviations: MACE, major adverse cardiovascular events; CV, cardiovascular; HHF, hospitalization for heart failure; FS, fibrosis score; FS_{0-2}, F0 – F2 fibrosis; FS+, indeterminant range or F3 – F4 fibrosis; HR, hazard ratio; CI, confidence interval.
Cox model interaction p-values of HR indicate differences by FS category in the effect of apabetalone on HR.
Categorical variables are presented as n (%).
P-values for categorical variables were calculated using chi-square test.

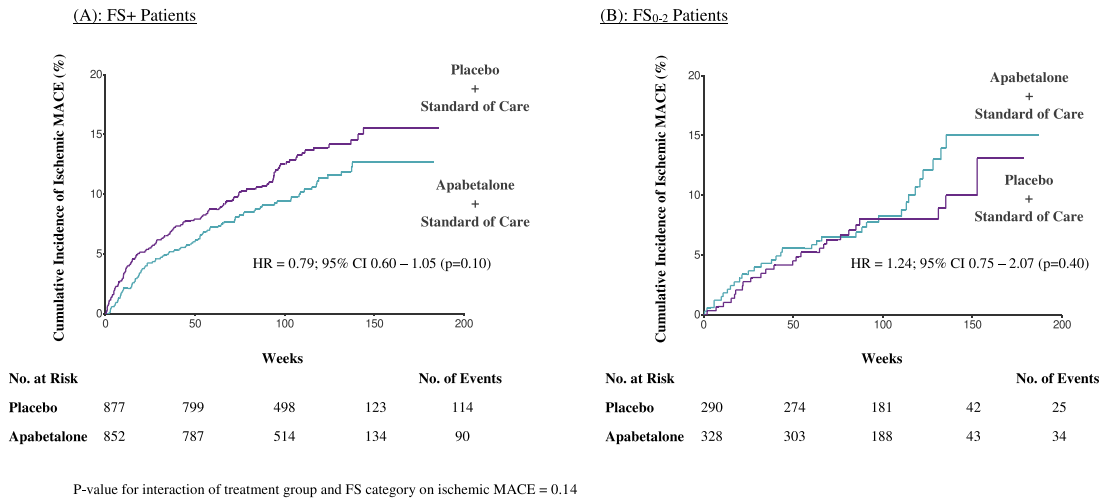


Fig. 2. Kaplan-Meier estimate of time to first occurrence of ischemic MACE (CV death, non-fatal MI, or stroke) in (A): FS+ patients; and, (B): FS_{0-2} patients. A log-rank test was used for the formal hypothesis testing. A Cox proportional hazards model was used to estimate the HR with 95% CI. P-values of <0.05 are considered statistically significant.

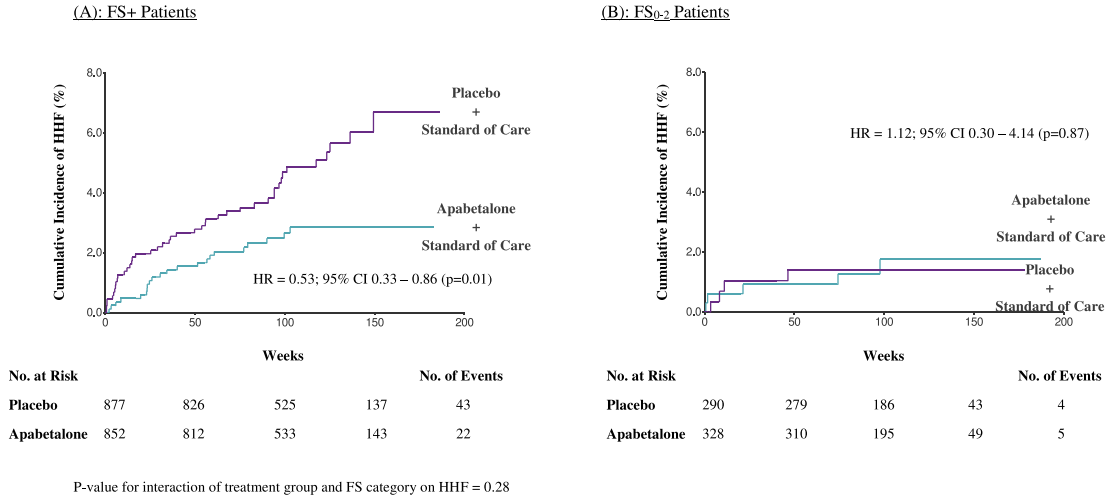


Fig. 3. Kaplan-Meier estimate of time to first occurrence of HHF in (A): FS+ patients; and, (B): FS_{0-2} patients. A log-rank test was used for the formal hypothesis testing. A Cox proportional hazards model was used to estimate the HR with 95% CI. P-values of <0.05 are considered statistically significant.

ACS T2DM patients are likely to have NAFLD. Strikingly, 73.7% of patients with T2DM and recent ACS had scores consistent with a moderate to high likelihood of advanced liver fibrosis, while only 26.3% had scores consistent with a low likelihood of advanced fibrosis [12]. Moreover, the baseline Angulo FS identified patients who were at high risk for further CV events despite proven, standard-of-care therapies. Importantly, patients with an elevated baseline FS were at a higher risk of ischemic MACE and HHF and showed a CV benefit from treatment with apabetalone, while no such benefit was apparent among those with lower baseline FS.

With recognition that the interaction of treatment and FS category on the composite of ischemic MACE and HHF was not statistically significant, a greater treatment benefit of apabetalone in FS+ patients is plausible because FS+ patients were older, had a higher prevalence of hypertension, and had a longer duration of diabetes. Each of these characteristics is associated both with elevated CV risk and with increased BET dysregulation and heightened response to BET-inhibition treatment [25–30].

Several studies have found that high FS associates with incidence of coronary heart disease [31,32] as well as the angiographic complexity [33–35], risk of MACE [34,36], and death [37] in patients with established coronary heart disease. In an analysis of the IMPROVE-IT trial that compared ezetimibe with placebo in patients with ACS treated with simvastatin [36], high FS (>0.67, n=2,106) vs low FS (<-1.455, n=5,440) was associated with 30% increased risk of MACE. Analogous to the findings for apabetalone in the current analysis, the data from IMPROVE-IT suggested that high FS was an effect modifier for the benefit of ezetimibe. In patients with high FS, ezetimibe conferred a 3.7% absolute reduction in risk of MACE compared to placebo (HR 0.85 [0.74-0.98]), while no benefit of ezetimibe was evident in the low FS group (HR 1.01 [0.91-1.12]; p-interaction = 0.053) [36].

The observed benefit of apabetalone in patients with high FS, serving as a surrogate for NAFLD, may be important as the already-high prevalence of NAFLD is predicted to increase across populations in the future, augmenting the need for effective, targeted treatments [38–43]. Although no drugs are currently approved for treating NAFLD, PPAR activators, which may also favorably influence a dysregulated transcriptional profile, have shown promise [44,45]. In addition, exercise, weight loss, and bariatric surgery have been shown to regress steatosis or even resolve distorted hepatic cellular architecture [46–50]. Considerable investigation is ongoing worldwide to find safe and efficacious therapies to treat NAFLD so as to prevent hepatic injury and

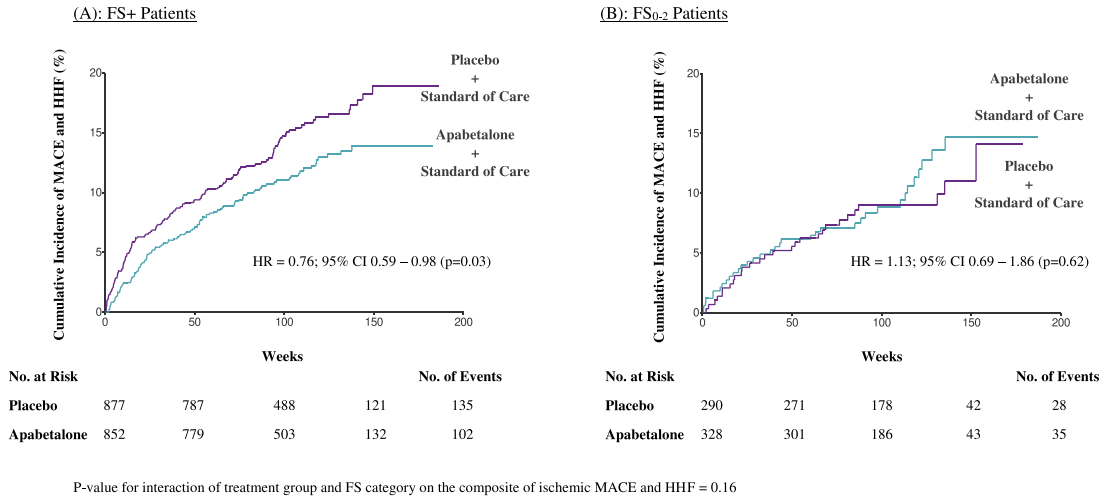


Fig. 4. Kaplan-Meier estimate of time to first occurrence of the composite of ischemic MACE (CV death, non-fatal MI, or stroke) and HHF in (A): FS+ patients; and, (B): FS_{0-2} patients. A log-rank test was used for the formal hypothesis testing. A Cox proportional hazards model was used to estimate the HR with 95% CI. P-values of <0.05 are considered statistically significant.

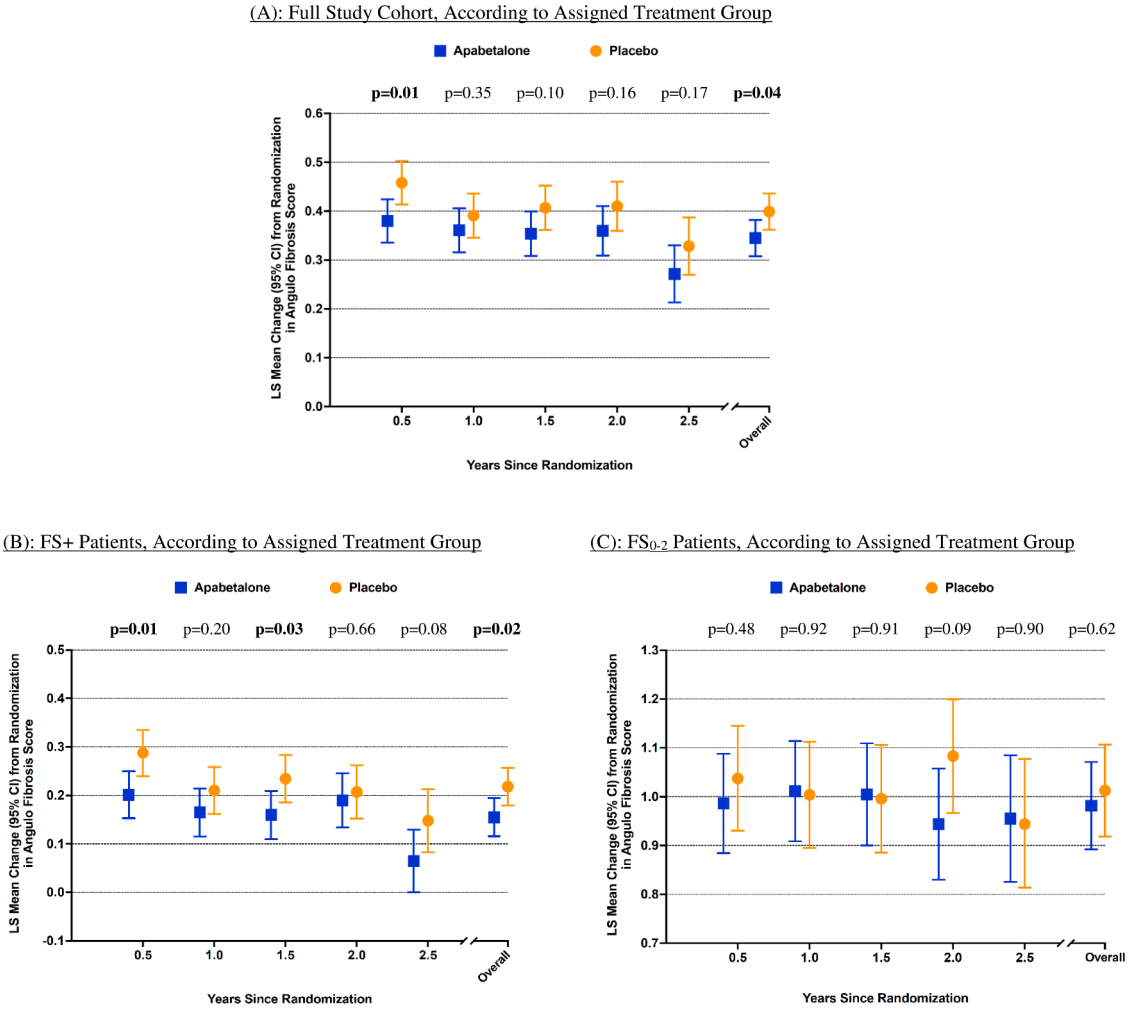


Fig. 5. Change in FS over time in (A): the full study cohort, according to assigned treatment group; (B): FS+ patients, according to assigned treatment group; and, (C): FS_{0-2} patients, according to assigned treatment group. Change in FS over was analyzed using a repeated-measures mixed-effects model with absolute change from baseline as the outcome, random effects for intercept and baseline FS score, and fixed effects for treatment group and time, modeled jointly with time to all-cause death as a sensitivity to account for competing risk. Data are presented as least squares (LS) means with 95% confidence intervals (CI). LS means and p-values at each timepoint were determined by interaction terms between treatment and time. P-values of <0.05 are considered statistically significant.

Table 4
Adverse events (AEs) according to assigned treatment group, and FS category. Data are presented as n (%).

	Full Study Cohort According to Assigned Treatment Group		Full Study Cohort According to FS Category	
	Placebo (n=1,167)	Apabetalone (n=1,180)	FS_{0-2} Patients (n=618)	FS+ Patients (n=1,729)
Patients with at least one adverse event*	792 (68%)	813 (69%)	428 (69%)	1,177 (68%)
Patients with at least one serious adverse event*	324 (28%)	346 (29%)	158 (26%)	512 (30%)
Patients with at least one adverse event leading to study drug discontinuation*	65 (5.6%)	110 (9.3%)	42 (6.8%)	133 (7.7%)
Frequent adverse events*,†				
Alanine aminotransferase increased	17 (1.5%)	63 (5.3%)	23 (3.7%)	57 (3.3%)
Angina pectoris	72 (6.2%)	72 (6.1%)	35 (5.7%)	109 (6.3%)
Aspartate aminotransferase increased	8 (0.7%)	20 (1.7%)	5 (0.8%)	23 (1.3%)
Diabetes mellitus	58 (5.0%)	75 (6.4%)	35 (5.7%)	98 (5.7%)
Diarrhea	41 (3.5%)	42 (3.6%)	31 (5.0%)	52 (3.0%)
Gamma-glutamyltransferase increased	12 (1.0%)	10 (0.8%)	4 (0.6%)	18 (1.0%)
Hypertension	70 (6.0%)	68 (5.8%)	32 (5.2%)	106 (6.1%)

* Includes treatment-emergent adverse events only, defined as those occurring after the first dose and within 14 days of the last dose of the study drug.
† Defined as occurring with a frequency of 5% or more in any of the FS categories or treatment groups.

Table 5
Adverse events (AEs) according to FS category and assigned treatment group. Data are presented as n (%).

	FS_{0-2} Patients According to Assigned Treatment Group		FS+ Patients According to Assigned Treatment Group	
	Placebo (n=290)	Apabetalone (n=328)	Placebo (n=877)	Apabetalone (n=852)
Patients with at least one adverse event*	205 (71%)	223 (68%)	587 (67%)	590 (69%)
Patients with at least one serious adverse event*	72 (25%)	86 (26%)	252 (29%)	260 (31%)
Patients with at least one adverse event leading to study drug discontinuation*	13 (4.5%)	29 (8.8%)	52 (5.9%)	81 (9.5%)
Frequent adverse events*,†				
Alanine aminotransferase increased	4 (1.4%)	19 (5.8%)	13 (1.5%)	44 (5.2%)
Angina pectoris	21 (7.2%)	14 (4.3%)	51 (5.8%)	58 (6.8%)
Angina unstable	6 (2.1%)	18 (5.5%)	33 (3.8%)	38 (4.5%)
Aspartate aminotransferase increased	2 (0.7%)	3 (0.9%)	6 (0.7%)	17 (2.0%)
Diabetes mellitus	16 (5.5%)	19 (5.8%)	42 (4.8%)	56 (6.6%)
Diarrhea	13 (4.5%)	18 (5.5%)	28 (3.2%)	24 (2.8%)
Gamma-glutamyltransferase increased	2 (0.7%)	2 (0.6%)	10 (1.1%)	8 (0.9%)

* Includes treatment-emergent adverse events only, defined as those occurring after the first dose and within 14 days of the last dose of the study drug.
† Defined as occurring with a frequency of 5% or more in any of the FS categories or treatment groups.

attenuate risk for CVD. Many classes of drugs are being tested for efficacy and safety [51–53].

The observation that FS was lower under treatment with the BET protein inhibitor apabetalone compared with placebo in FS+ patients is novel. However, the difference in FS between treatment groups was small. Further studies are needed to determine if a favorable effect of apabetalone on FS is accompanied by favorable changes in hepatic imaging, mechanical properties, or histology, whether parallel reductions in steatosis are observed in extra-hepatic tissues including the heart [54,55], in turn whether reducing steatosis-driven inflammation attenuates the risk of CVD events [56,57].

Epigenetic BET-inhibition by apabetalone may constitute a novel approach to slow or even stop the progression of hepatic steatosis and fibrosis, and may explain the CVD reduction by apabetalone observed in the FS+ category. Inflammatory stimuli drive human hepatocyte overexpression of acute phase reactants *in vitro*, creating a positive feedback loop that can promote local liver and systemic inflammation. By preventing BET protein – chromatin associations at specific translational start sites, apabetalone can reduce the transcription of multiple induced APR genes [18]. Additionally, it has been shown that the expression of key APR genes is lower in the liver of endotoxemic mice and in the plasma of coronary artery disease (CAD) patients following apabetalone treatment [18]. Furthermore, the abundance of pro-inflammatory mediators in the plasma of patients from multiple phase 2 clinical studies, is significantly lower in CAD patients treated with apabetalone [14,15,18,58]. Plasma ALP, sourced primarily from the liver, is a robust and independent predictor of all-cause mortality, as it promotes vascular calcification and arterial stiffness, vascular inflammation, destabilization of atherosclerotic plaques, and oxidative stress. Apabetalone has also been shown to reduce hepatocyte ALP *in vitro* [13], and ALP protein levels are reduced in CAD and chronic kidney disease (CKD) patient plasma following apabetalone treatment [58,59]. Finally, the BETi I-BET151 has been shown to improve NASH and liver fibrosis in the STAM mouse NASH model [60]. Thus, precedence exists for BETi efficacy in NASH, and apabetalone itself is predicted to benefit liver function through its epigenetic regulation of dysfunctional transcription that arises in NAFLD and CAD.

There are several limitations to this study. First, we used a validated scoring system based on six demographic and laboratory parameters that has been shown to associate with the prevalence of NAFLD determined by biopsy, imaging, or non-invasive biomechanical properties [12]. Based on a validation group of 253 subjects with NAFLD and advanced fibrosis, the Angulo model has a positive predictive value of 82% to diagnose fibrosis and a negative predictive value of 88% to exclude fibrosis [12]. However, we did not obtain imaging or biomechanical data to indicate whether the FS categories defined in this cohort corresponded to differences in measures of liver fibrosis or steatosis. Second, substantial data associate FS with the prevalence of NAFLD, but it is less clear whether changes in FS reflect changes in the clinical course of NAFLD. Accordingly, it is uncertain whether the acute improvement in FS with apabetalone compared with placebo reflects modulation of the underlying pathologic processes leading to NAFLD and its progression, or simply modulation of the biochemical contributors to FS. To make this distinction would require correlation of FS with concurrent hepatic histology, imaging, or biomechanics. Third, the number of patients with FS score consistent with low likelihood of NAFLD was relatively small and the assessment of treatment effect by apabetalone vs. placebo in this group may therefore have been underpowered. Finally,

this analysis is *post-hoc* and therefore should be considered hypothesis-generating. The primary clinical outcome of the BETonMACE trial did not attain statistical significance, so any inference of efficacy in a subgroup must be viewed as exploratory. Notwithstanding these limitations, the CVD reduction observed in the FS+ category is consistent with apabetalone's BET-inhibition mode of action. A prospective, placebo-controlled evaluation of hepatic and CV effects of apabetalone in patients with NAFLD is warranted.

CRediT authorship contribution statement

Peter P. Toth: Writing – original draft. **Gregory G. Schwartz:** Writing – original draft. **Stephen J. Nicholls:** Writing – review & editing. **Aziz Khan:** Formal analysis. **Michael Szarek:** Formal analysis. **Henry N. Ginsberg:** Writing – review & editing. **Jan O. Johansson:** Writing – original draft. **Kamyar Kalantar-Zadeh:** Writing – review & editing. **Ewelina Kulikowski:** Writing – review & editing. **Ken Lebioda:** Writing – review & editing. **Norman C.W. Wong:** Writing – review & editing. **Michael Sweeney:** Writing – review & editing. **Kausik K. Ray:** Writing – review & editing.

Declaration of Competing Interest

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Supplementary materials

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References

[1] Mahfood HT, Hamdeh S, Kanmanthareddy A, Alla VM. Nonalcoholic fatty liver disease and the risk of clinical cardiovascular events: A systematic review and meta-analysis. Diabetes Metab Syndr 2017;11(Suppl 1):S209–16.

[2] Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30(5):1212–8.

[3] Santos RD, Nasir K, Conceição RD, Sarwar A, Carvalho JA, Blumenthal RS. Hepatic steatosis is associated with a greater prevalence of coronary artery calcification in asymptomatic men. Atherosclerosis 2007;194(2):517–9.

[4] Akhtar DH, Iqbal U, Vazquez-Montesino LM, Dennis BB, Ahmed A. Pathogenesis of insulin resistance and atherogenic dyslipidemia in nonalcoholic fatty liver disease. J Clin Transl Hepatol 2019;7(4):362–70.

[5] Chen Z, Tian R, She Z, Cai J, Li H. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. Free Radic Biol Med 2020;152:116–41.

[6] Taipale T, Seppälä I, Raitoharju E, et al. Fatty liver is associated with blood pathways of inflammatory response, immune system activation and prothrombotic state in Young Finns Study. Sci Rep 2018;8(1):10358.

[7] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 2010;51(2):679–89.

[8] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus-mechanisms and treatments. Nat Rev Gastroenterol Hepatol 2021;18(9):599–612.

[9] Tana C, Ballestri S, Ricci F, et al. Cardiovascular risk in non-alcoholic fatty liver disease: mechanisms and therapeutic implications. Int J Environ Res Public Health 2019;16(17):3104.

[10] Bertot LC, Adams LA. The natural course of non-alcoholic fatty liver disease. Int J Mol Sci 2016;17(5):774.

[11] Toth PP. Epicardial steatosis, insulin resistance, and coronary artery disease. Heart Fail Clin 2012;8(4):671–8.

[12] 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(4):846–54.

[13] Gilham D, Tsujikawa LM, Sarsons CD, et al. Apabetalone downregulates factors and pathways associated with vascular calcification. Atherosclerosis 2019;280:75–84.

[14] Tsujikawa LM, Fu L, Das S, et al. Apabetalone (RVX-208) reduces vascular inflammation *in vitro* and in CVD patients by a BET-dependent epigenetic mechanism. Clin Epigenet 2019;11(1):102.

[15] Wong NCW, Kulikowski E, Wasiak S, et al. Apabetalone (RVX-208) decreases atherogenic, thrombotic and inflammatory mediators *in vitro* and in plasma of patients with cardiovascular disease (CVD). Atherosclerosis 2016;252:e245 (Abstract).

[16] Wasiak S, Gilham D, Tsujikawa LM, et al. Data on gene and protein expression changes induced by apabetalone (RVX-208) in *ex vivo* treated human whole blood and primary hepatocytes. Data Brief 2016;8:1280–8.

[17] Gilham D, Wasiak S, Tsujikawa LM, et al. RVX-208, a BET-inhibitor for treating atherosclerotic cardiovascular disease, raises ApoA-I/HDL and represses pathways that contribute to cardiovascular disease. Atherosclerosis 2016;247:48–57.

[18] Wasiak S, Gilham D, Daze E, et al. Epigenetic modulation by apabetalone counters cytokine-driven acute phase response *in vitro*, in mice and in patients with cardiovascular disease. Cardiovasc Ther 2020;2020:9397109.

[19] Ray KK, Nicholls SJ, Buhr KA, et al. Effect of apabetalone added to standard therapy on major adverse cardiovascular events in patients with recent acute coronary syndrome and type 2 diabetes: a randomized clinical trial. JAMA 2020;323(16):1565–73.

[20] Ray KK, Nicholls SJ, Ginsberg HD, et al. Effect of selective BET protein inhibitor apabetalone on cardiovascular outcomes in patients with acute coronary syndrome and diabetes: Rationale, design, and baseline characteristics of the BETonMACE trial. Am Heart J 2019;217:72–83.

[21] Nicholls SJ, Schwartz GG, Buhr KA, et al. Apabetalone and hospitalization for heart failure in patients following an acute coronary syndrome: a prespecified analysis of the BETonMACE study. Cardiovasc Diabetol 2021;20(1):13.

[22] Kalantar-Zadeh K, Schwartz GG, Nicholls SJ, et al. Effect of apabetalone on cardiovascular events in diabetes, CKD, and recent acute coronary syndrome: results from the BETonMACE randomized controlled trial. Clin J Am Soc Nephrol 2021;16(5):705–16.

[23] Schwartz GG, Nicholls SJ, Toth PP, et al. Relation of insulin treatment for type 2 diabetes to the risk of major adverse cardiovascular events after acute coronary syndrome: an analysis of the BETonMACE randomized clinical trial. Cardiovasc Diabetol 2021;20(1):125.

[24] Cummings J, Schwartz GG, Nicholls SJ, et al. Cognitive effects of the BET protein inhibitor apabetalone: a prespecified montreal cognitive assessment analysis nested in the BETonMACE randomized controlled trial. J Alzheimers Dis 2021;83(4):1703–15.

[25] Wasiak S, Dzobo KE, Rakai BD, et al. BET protein inhibitor apabetalone (RVX-208) suppresses pro-inflammatory hyper-activation of monocytes from patients with cardiovascular disease and type 2 diabetes. Clin Epigenet 2020;12(1):166.

[26] Borck PC, Guo LW, Plutzky J. BET epigenetic reader proteins in cardiovascular transcriptional programs. Circ Res 2020;126(9):1190–208.

[27] Neele AE, Willemsen L, Chen HJ, Dzobo KE, de Winther MPJ. Targeting epigenetics as atherosclerosis treatment: an updated view. Curr Opin Lipidol 2020;31(6):324–30.

[28] Meloche J, Potus F, Vaillancourt M, et al. Bromodomain-containing protein 4: the epigenetic origin of pulmonary arterial hypertension. Circ Res 2015;117(6):525–35.

[29] Van der Feen DE, Kurakula K, Tremblay E, et al. Multicenter preclinical validation of BET inhibition for the treatment of pulmonary arterial hypertension. Am J Respir Crit Care Med 2019;200(7):910–20.

[30] Nicholas DA, Andrieu G, Strissel KJ, Nikolajczyk BS, Denis GV. BET bromodomain proteins and epigenetic regulation of inflammation: implications for type 2 diabetes and breast cancer. Cell Mol Life Sci 2017;74(2):231–43.
[31] Schonmann Y, Yeshua H, Bentov I, Zelber-Sagi S. Liver fibrosis marker is an independent predictor of cardiovascular morbidity and mortality in the general population. Dig Liver Dis 2021;53(1):79–85.
[32] Dogan S, Celikbilek M, Yilmaz YK, et al. Association between liver fibrosis and coronary heart disease risk in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2015;27(3):298–304.
[33] Jin JL, Zhang HU, Cao YX, et al. Liver fibrosis scores and coronary atherosclerosis: novel findings in patients with stable coronary artery disease. Hepatol Int 2021;15(2):413–23.
[34] Turan Y. The nonalcoholic fatty liver disease fibrosis score is related to epicardial fat thickness and complexity of coronary artery disease. Angiology 2020;71(1):77–82.
[35] Chen LZ, Jing XB, Wu CF, et al. Nonalcoholic fatty liver disease-associated liver fibrosis is linked with the severity of coronary artery disease mediated by systemic inflammation. Dis Markers 2021;2021:6591784.
[36] Simon TG, Corey KE, Cannon CP, et al. The nonalcoholic fatty liver disease (NAFLD) fibrosis score, cardiovascular risk stratification and a strategy for secondary prevention with ezetimibe. Int J Cardiol 2018;270:245–52.
[37] Chen Q, Li Q, Li D, et al. Association between liver fibrosis scores and the risk of mortality among patients with coronary artery disease. Atherosclerosis 2020;299:45–52.
[38] Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol 2019;71(4):793–801.
[39] Kasper P, Martin A, Lang S, et al. NAFLD and cardiovascular diseases: a clinical review. Clin Res Cardiol 2021;110(7):921–37.
[40] Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. Mitochondrion 2006;6(1):1–28.
[41] Reibe S, Febbraio MA. Relieving ER stress to target NASH-driven hepatocellular carcinoma. Nat Rev Endocrinol 2019;15(2):73–4.
[42] Arroyave-Ospina JC, Wu Z, Geng Y, Moshage H. Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy. Antioxidants 2021;10(2):174 (Basel).
[43] Masarone M, Rosato V, Dallio M, et al. Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. Oxid Med Cell Longev 2018;2018:9547613.
[44] Boeckmans J, Natale A, Rombaut M, et al. Anti-NASH drug development hitches a lift on PPAR Agonism. Cells 2019;9(1):37.
[45] Francque SM, Bedossa P, Ratziu V, et al. A randomized, controlled trial of the Pan-PPAR agonist lanifibranor in NASH. N Engl J Med 2021;385(17):1547–58.
[46] Armandi A, Schattenberg JM. Beyond the paradigm of weight loss in non-alcoholic fatty liver disease: from pathophysiology to novel dietary approaches. Nutrients 2021;13(6):1977.
[47] Nakao Y, Amrollahi P, Parthasarathy G, et al. Circulating extracellular vesicles are a biomarker for NAFLD resolution and response to weight loss surgery. Nanomedicine 2021;36:102430.
[48] Tacke F, Weiskirchen R. Non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH)-related liver fibrosis: mechanisms, treatment and prevention. Ann Transl Med 2021;9(8):729.
[49] Holmer M, Lindqvist C, Petersson S, et al. Treatment of NAFLD with intermittent calorie restriction or low-carb high-fat diet - a randomised controlled trial. JHEP Rep 2021;3(3):100256.
[50] Koutoukidis DA, Jebb SA, Tomlinson JW, Cobbold JF, Aveyard P. Association of weight changes with changes in histological features and blood markers in nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2022;20(3):e538–47.
[51] Matye DJ, Wang H, Luo W, et al. Combined ASBT inhibitor and FGF15 treatment improves therapeutic efficacy in experimental nonalcoholic steatohepatitis. Cell Mol Gastroenterol Hepatol 2021;12(3):1001–19.
[52] Dichtel LE. The glucagon-like peptide-1 receptor agonist, semaglutide, for the treatment of nonalcoholic steatohepatitis. Hepatology 2021;74(4):2290–2.
[53] Vuppalanchi R, Noureddin M, Alkhouri N, Sanyal AJ. Therapeutic pipeline in nonalcoholic steatohepatitis. Nat Rev Gastroenterol Hepatol 2021;18(6):373–92.
[54] Pezzilli R, Calculli L. Pancreatic steatosis: is it related to either obesity or diabetes mellitus? World J Diabetes 2014;5(4):415–9.
[55] Conceição G, Martins D, Miranda IM, Leite-Moreira AF, Vitorino R, Falcão-Pires I. Unraveling the role of epicardial adipose tissue in coronary artery disease: partners in crime? Int J Mol Sci 2020;21(22):8866.
[56] Perseghin G. Lipids in the wrong place: visceral fat and nonalcoholic steatohepatitis. Diabetes Care 2011;34(Suppl 2):S367–70. Suppl 2.
[57] Fitzgibbons TP, Czech MP. Epicardial and perivascular adipose tissues and their influence on cardiovascular disease: basic mechanisms and clinical associations. J Am Heart Assoc 2014;3(2):e000582.
[58] Haarhaus M, Ray KK, Nicholls SJ, et al. Apabetalone lowers serum alkaline phosphatase and improves cardiovascular risk in patients with cardiovascular disease. Atherosclerosis 2019;290:59–65.
[59] Kulikowski E, Halliday C, Johansson J, et al. Apabetalone mediated epigenetic modulation is associated with favorable kidney function and alkaline phosphatase profile in patients with chronic kidney disease. Kidney Blood Press Res 2018;43(2):449–57.
[60] Middleton SA, Rajpal N, Cutler L, et al. BET inhibition improves NASH and liver fibrosis. Sci Rep 2018;8(1):17257.