A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis

NN9931-4296 Investigators

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ORIGINAL ARTICLE

A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis

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ABSTRACT

BACKGROUND

Nonalcoholic steatohepatitis (NASH) is a common disease that is associated with increased morbidity and mortality, but treatment options are limited. The efficacy and safety of the glucagon-like peptide-1 receptor agonist semaglutide in patients with NASH is not known.

METHODS

We conducted a 72-week, double-blind phase 2 trial involving patients with biopsy-confirmed NASH and liver fibrosis of stage F1, F2, or F3. Patients were randomly assigned, in a 3:3:3:1:1:1 ratio, to receive once-daily subcutaneous semaglutide at a dose of 0.1, 0.2, or 0.4 mg or corresponding placebo. The primary end point was resolution of NASH with no worsening of fibrosis. The confirmatory secondary end point was an improvement of at least one fibrosis stage with no worsening of NASH. The analyses of these end points were performed only in patients with stage F2 or F3 fibrosis; other analyses were performed in all the patients.

RESULTS

In total, 320 patients (of whom 230 had stage F2 or F3 fibrosis) were randomly assigned to receive semaglutide at a dose of 0.1 mg (80 patients), 0.2 mg (78 patients), or 0.4 mg (82 patients) or to receive placebo (80 patients). The percentage of patients in whom NASH resolution was achieved with no worsening of fibrosis was 40% in the 0.1-mg group, 36% in the 0.2-mg group, 59% in the 0.4-mg group, and 17% in the placebo group ($P<0.001$ for semaglutide 0.4 mg vs. placebo). An improvement in fibrosis stage occurred in 43% of the patients in the 0.4-mg group and in 33% of the patients in the placebo group ($P=0.48$). The mean percent weight loss was 13% in the 0.4-mg group and 1% in the placebo group. The incidence of nausea, constipation, and vomiting was higher in the 0.4-mg group than in the placebo group (nausea, 42% vs. 11%; constipation, 22% vs. 12%; and vomiting, 15% vs. 2%). Malignant neoplasms were reported in 3 patients who received semaglutide (1%) and in no patients who received placebo. Overall, neoplasms (benign, malignant, or unspecified) were reported in 15% of the patients in the semaglutide groups and in 8% in the placebo group; no pattern of occurrence in specific organs was observed.

CONCLUSIONS

This phase 2 trial involving patients with NASH showed that treatment with semaglutide resulted in a significantly higher percentage of patients with NASH resolution than placebo. However, the trial did not show a significant between-group difference in the percentage of patients with an improvement in fibrosis stage. (Funded by Novo Nordisk; ClinicalTrials.gov number, NCT02970942.)

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*A complete list of the investigators in this trial is provided in the Supplementary Appendix, available at NEJM.org.

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Nonalcoholic steatohepatitis (NASH) is a severe form of nonalcoholic fatty liver disease that is characterized by the accumulation of fat (steatosis), hepatocyte damage, and inflammation. It can be associated with fibrosis, cirrhosis, and an increased risk of hepatocellular carcinoma, cardiovascular disease, chronic kidney disease, and death.[1-3]

Insulin resistance is a shared characteristic of type 2 diabetes and obesity and is a key pathogenic driver of NASH.[4-6] Increased adiposity, adipose tissue dysfunction, and insulin resistance contribute to increased levels of free fatty acids and carbohydrates, which place excess lipotoxic and metabolic loads on the liver and ultimately lead to hepatic lipid accumulation, cell injury, inflammation, and fibrosis.[4,6] To date, there are no approved pharmacotherapies for the treatment of NASH. Pioglitazone and vitamin E may be considered as possible treatment options with management focused on lifestyle interventions to encourage weight loss and treatment of coexisting conditions.[1,7,8]

The glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide has been shown to improve liver-enzyme levels and reduce liver fat[9] and to have a beneficial effect on histologic resolution of NASH.[10] Semaglutide is another GLP-1 receptor agonist; it is approved for the treatment of type 2 diabetes[11] and is being studied for use in weight management.[12] Semaglutide has a mechanism of action that is similar to that of liraglutide but with more pronounced metabolic effects.[13-15] In previous studies, semaglutide induced weight loss and improved glycemic control in patients with obesity[15] and type 2 diabetes[16] and was associated with reduced cardiovascular risk among patients with type 2 diabetes at high cardiovascular risk.[17] Moreover, semaglutide has been reported to reduce levels of alanine aminotransferase and markers of inflammation.[18] We conducted a randomized, placebo-controlled, phase 2 trial to investigate the effect of semaglutide on histologic resolution of NASH in patients with biopsy-confirmed NASH and fibrosis.

Methods

Trial Design and Oversight

This randomized, double-blind, placebo-controlled, parallel-group trial was conducted at 143 sites in 16 countries. The trial consisted of a 72-week treatment period and a 7-week follow-up period. The protocol, which is available with the full text of this article at NEJM.org, was approved by the institutional review board and ethics committee at each participating trial site. All the patients provided written informed consent.

The sponsor (Novo Nordisk) designed the trial and performed site monitoring, data collection, and data analysis. All the authors had access to the data, participated in data interpretation, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. An earlier version of the manuscript was drafted with the assistance of medical writers (funded by the sponsor), under the guidance of the authors.

Patients

Eligible patients were 18 to 75 years of age (20 to 75 years of age in Japan), with or without type 2 diabetes, and had a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) of greater than 25 at screening. Additional key inclusion criteria were histologic evidence of NASH and an activity score for nonalcoholic fatty liver disease[19] of 4 or higher, with a subscore of 1 or higher for each subcomponent (steatosis, hepatocyte ballooning, and lobular inflammation) and a fibrosis stage[19] of F2 or F3 (amended to F1, F2, or F3 during the trial).

Key exclusion criteria were a glycated hemoglobin level of greater than 9.5% at screening (amended to >10% during the trial), causes of chronic liver disease other than NASH, excessive alcohol consumption (>20 g per day for women; >30 g per day for men), and confounding concomitant drug use (including vitamin E or treatment with pioglitazone if the patient was not receiving a stable dose). Full eligibility criteria are listed in the Supplementary Appendix, available at NEJM.org.

Procedures

Patients were randomly assigned, in a 3:3:3:1:1:1 ratio, to receive once-daily subcutaneous semaglutide at a dose of 0.1, 0.2, or 0.4 mg or corresponding placebo (with blinding of treatment assignments within dose levels) (Fig. S1 in the Supplementary Appendix). Randomization was performed with the use of an interactive Web-response system and stratified according to

geographic region (Japan vs. all other regions), type 2 diabetes status (with vs. without), and fibrosis stage (stage F1 or F2 vs. stage F3). Once-daily semaglutide was initiated at a dose of 0.05 mg, then increased to 0.1 mg after 4 weeks, and subsequently increased by 0.1 mg every 4 weeks thereafter, until the randomly assigned dose was reached. Dose adjustment was not permitted once the target dose was reached; patients who had unacceptable side effects from the randomly assigned dose discontinued treatment but were requested to continue with scheduled site visits until week 72. Throughout the trial, patients received counseling regarding nutrition and physical activity, in accordance with local practice.

Screening biopsy results were used as baseline for histologic variables, and an additional biopsy was performed at week 72. Each biopsy was assessed centrally by two independent expert hepatopathologists in a sequential manner to determine the activity score for nonalcoholic fatty liver disease and the fibrosis stage (according to NASH Clinical Research Network criteria). The pathologists were unaware of the treatment assignments, patient characteristics, and each other's assessments. The two pathologists agreed on all the variables (steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis stage) in 24% of the assessments (with 62 to 75% agreement on individual components). In cases of discordant assessment on any variable, agreement was achieved through a consensus call; details are provided in the Supplementary Appendix.

END POINTS

The primary end point was resolution of NASH (defined by the NASH Clinical Research Network as no more than mild residual inflammatory cells [score of 0 or 1] and no hepatocyte ballooning [score of 0]) and no worsening of liver fibrosis (with worsening defined as an increase of one stage or more on the Kleiner fibrosis classification scale) after 72 weeks, in line with regulatory perspectives.[20,21] The confirmatory secondary end point (controlled for multiple comparisons) was an improvement of at least one fibrosis stage and no worsening of NASH (with worsening defined as an increase of ≥1 point in either the lobular inflammation score or the hepatocyte ballooning score according to the NASH Clinical Research Network criteria) after 72 weeks.

Supportive secondary histologic end points included the change from baseline to week 72 in fibrosis stage, total activity score for nonalcoholic fatty liver disease, and subscores of the components of nonalcoholic fatty liver disease (steatosis, hepatocyte ballooning, and lobular inflammation). Other secondary end points included changes from baseline to week 72 in serum liver-enzyme levels, exploratory biomarker levels (including the enhanced liver fibrosis test score), liver stiffness and steatosis as assessed by FibroScan (Echosens) (performed only at sites at which equipment was available), body weight, glucose metabolism, blood pressure, and lipid levels. Safety end points included adverse events after the start of treatment, biochemical assessments, and clinical assessments. Selected events (including deaths, cardiovascular events, and acute pancreatitis) were adjudicated by an independent, external event-adjudication committee, whose members were unaware of the treatment assignments. A complete list of the trial end points is provided in Table S1.

STATISTICAL ANALYSIS

The sample size was reduced during the trial on the basis of emerging placebo response data from other NASH trials. The final sample size was based on the assumption that 14% of the patients in the placebo group and 38% of the patients in the semaglutide 0.4-mg group would meet the criteria for the primary end point, after we accounted for a dropout rate of up to 15%. We determined that enrollment of 288 patients (including all those with eligible fibrosis stages) would be needed to evaluate the primary end point for the semaglutide 0.4-mg group with 90% power.

The efficacy analyses included all the patients who underwent randomization, whereas the safety analyses included patients who received at least one dose of semaglutide or placebo. Data from the three placebo groups were pooled.

The main analyses of the primary end point and the confirmatory secondary end point were performed in patients with stage F2 or F3 fibrosis at baseline (specified in the statistical analysis plan, which was written after recruitment had ended but before unblinding of the treatment assignments), to match the population considered by regulatory authorities to be the intended target population.[20,21] These analyses were per-

formed with the use of a Cochran–Mantel–Haenszel test, with adjustment for baseline diabetes status and baseline fibrosis stage. Patients with missing data were considered as not having had a response. A sensitivity analysis, in which missing data were alternatively handled by multiple imputation from the placebo group, was performed (details are provided in the Supplementary Appendix).

Multiplicity was addressed by a hierarchical testing procedure that included the primary end point and the confirmatory secondary end point (added to the hierarchy in the statistical analysis plan). The testing procedure first compared the semaglutide 0.4-mg group with the placebo group for the primary end point; if that comparison confirmed superiority, then these groups were compared for the confirmatory secondary end point. The comparisons for the primary and confirmatory secondary end points proceeded with the lower doses until superiority of semaglutide over placebo was not confirmed (see Section 2 in the statistical analysis plan).

Two-sided P values of less than 0.05 (equivalent to a one-sided level of 0.025) were considered to indicate statistical significance. In addition, 95% confidence intervals for odds ratios were calculated, but without adjustment for multiple comparisons. Therefore, inferences drawn from the confidence intervals may not be reproducible.

The supportive secondary end points were analyzed in the full trial population (i.e., patients with stage F1, F2, or F3 fibrosis) and the results are presented as point estimates with 95% confidence intervals, without adjustment for multiple comparisons. Continuous end points were analyzed with the use of an analysis-of-covariance model, with missing data imputed with the use of multiple imputation from the placebo group. Further details are provided in the statistical analysis plan.

RESULTS

PATIENTS

From January 2017 through September 2018, 320 patients were randomly assigned to receive once-daily semaglutide at a dose of 0.1 mg (80 patients), 0.2 mg (78 patients), or 0.4 mg (82 patients) or to receive placebo (80 patients). In total, 302 patients (94%) completed the trial (i.e., had their final scheduled visit) and 285 patients (89%) completed treatment (Fig. S2). Information for the primary and confirmatory secondary outcomes was available for 277 patients (87%) with a biopsy at week 72 that could be evaluated. For the remaining 43 patients (13%), these outcomes were imputed as nonresponse with the use of a multiple-imputation analysis (sensitivity analysis).

Demographic and baseline clinical characteristics were similar across the trial groups (Table 1 and Table S2). Most of the patients were White (78%), were women (61%), and had type 2 diabetes (62%). The mean age was 55 years, the mean body weight 98.4 kg, and the mean BMI 35.8. A total of 90 patients (28%) had stage F1 fibrosis, 72 (22%) had stage F2, and 158 (49%) had stage F3; the mean activity score for nonalcoholic fatty liver disease was 4.9.

EFFICACY

Among the patients with stage F2 or F3 fibrosis, the percentage of patients in whom NASH resolution was achieved with no worsening of fibrosis after 72 weeks (the primary end point) was significantly higher in the semaglutide groups than in the placebo group, with the highest percentage observed in the 0.4-mg group (59% in the 0.4-mg group vs. 17% in the placebo group; odds ratio, 6.87; 95% confidence interval [CI], 2.60 to 17.63; P<0.001) (Fig. 1A). Similar results were seen in the sensitivity analysis in which multiple imputation was used to account for missing data (62% in the semaglutide 0.4-mg group vs. 18% in the placebo group; odds ratio, 7.33; 95% CI, 2.92 to 18.44) (Fig. S3).

The difference between the semaglutide 0.4-mg group and the placebo group in the percentage of patients who had an improvement of at least one fibrosis stage without worsening of NASH after 72 weeks (the confirmatory secondary end point) was not significant (43% and 33%, respectively; odds ratio, 1.42; 95% CI, 0.62 to 3.28; P=0.48) (Fig. 1B). This was the second comparison in the hierarchical plan to adjust for multiple comparisons, and therefore, formal testing was not done for the comparisons between the placebo group and the other dose groups. The sensitivity analysis of the secondary confirmatory end point in which multiple imputation was used showed similar results (50% in the semaglutide 0.4-mg group and 36% in the placebo group; odds ratio, 1.67; 95% CI, 0.75 to 3.71)

Table 1. Demographic and Baseline Clinical Characteristics.*

Characteristic	Semaglutide 0.1-mg Group (N=80)	Semaglutide 0.2-mg Group (N=78)	Semaglutide 0.4-mg Group (N=82)	Placebo Group (N=80)
Age — yr	55.2±10.9	58.1±9.9	54.3±10.2	52.4±10.8
Female sex — no. (%)	51 (64)	52 (67)	47 (57)	44 (55)
Body weight — kg	98.4±21.1	97.1±22.0	96.6±20.1	101.3±23.3
Body-mass index	36.1±6.4	35.6±6.1	35.2±6.6	36.1±6.6
Type 2 diabetes — no. (%)	49 (61)	51 (65)	49 (60)	50 (62)
Glycated hemoglobin level among patients with type 2 diabetes — %†	7.4±1.3	7.2±1.0	7.2±1.2	7.3±1.2
Liver-enzyme levels — U/liter				
Alanine aminotransferase	55±90	53±78	54±84	55±92
Aspartate aminotransferase	44±82	43±73	44±78	42±83
Liver fibrosis stage — no. (%)‡				
F1	23 (29)	19 (24)	26 (32)	22 (28)
F2	18 (22)	18 (23)	14 (17)	22 (28)
F3	39 (49)	41 (53)	42 (51)	36 (45)
Total activity score for nonalcoholic fatty liver disease§	4.9±0.8	4.9±0.9	4.8±0.9	4.9±0.9
Noninvasive measures of liver steatosis and fibrosis				
Liver steatosis, as assessed by FibroScan — dB/m¶	332.0±46.2	347.4±55.0	335.7±55.8	348.6±35.2
Liver stiffness, as assessed by FibroScan — kPa¶	10.4±78.5	12.3±74.0	11.5±87.1	8.7±90.0
Enhanced liver fibrosis test score‖	9.8±1.0	9.8±0.9	9.9±1.0	9.6±0.9

* Plus–minus values are means ±SD, except for body-mass index, liver-enzyme levels, and liver stiffness as assessed by FibroScan, which are geometric means ±coefficient of variation. Percentages may not total 100 because of rounding.
† These values were based on the number of patients with type 2 diabetes in each group (49, 51, 49, and 50 patients in the 0.1-mg, 0.2-mg, 0.4-mg, and placebo groups, respectively).
‡ Stages are defined as follows: F0, no fibrosis; F1, mild-to-moderate zone 3 perisinusoidal fibrosis or portal or periportal fibrosis only; F2, zone 3 perisinusoidal fibrosis and portal or periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis.
§ Scores range from 0 to 8 (unweighted sum of the scores for steatosis [assessed on a scale of 0 to 3], lobular inflammation [assessed on a scale of 0 to 3], and hepatocyte ballooning [assessed on a scale of 0 to 2]), with higher scores indicating an increased likelihood of nonalcoholic steatohepatitis.[19]
¶ This assessment was performed only at sites at which FibroScan equipment was available. Liver steatosis was assessed in 161 patients and liver stiffness in 212 patients.
‖ The enhanced liver fibrosis test provides an algorithmic liver fibrosis score that is based on the serum levels of hyaluronic acid, procollagen type III N-terminal peptide, and tissue inhibitor of metalloproteinase 1. A score of greater than 9.8 indicates a moderate risk of advanced fibrosis, and a score of greater than 11.3 denotes a high risk of advanced fibrosis.

(Fig. S4). An improvement of at least two fibrosis stages occurred in 25% of the patients in the semaglutide 0.1-mg group, in 19% in the 0.2-mg group, in 20% in the 0.4-mg group, and in 17% in the placebo group.

The results of the analyses that included all the patients who underwent randomization (any fibrosis stage) were consistent with those of the main analysis (which included patients with stage F2 or F3 fibrosis only) for both end points (Table S3 and Fig. S5). The results for both end points among patients with or without type 2 diabetes were similar (Fig. S6).

Among all the patients who underwent randomization, worsening of fibrosis occurred in 10%, 8%, and 5% of the patients in the semaglutide 0.1-mg, 0.2-mg, and 0.4-mg groups, respectively, and in 19% of the patients in the placebo group (Fig. S7). Progression to fibrosis stage F4 occurred in 3%, 3%, 0, and 4% of the patients, respectively (Table S4). The percentage of patients who had both NASH resolution and an improvement in fibrosis stage was 37% in the semaglutide 0.4-mg group and 15% in the placebo group. Improvement (of ≥1 point) in the nonalcoholic fatty liver disease activity score was

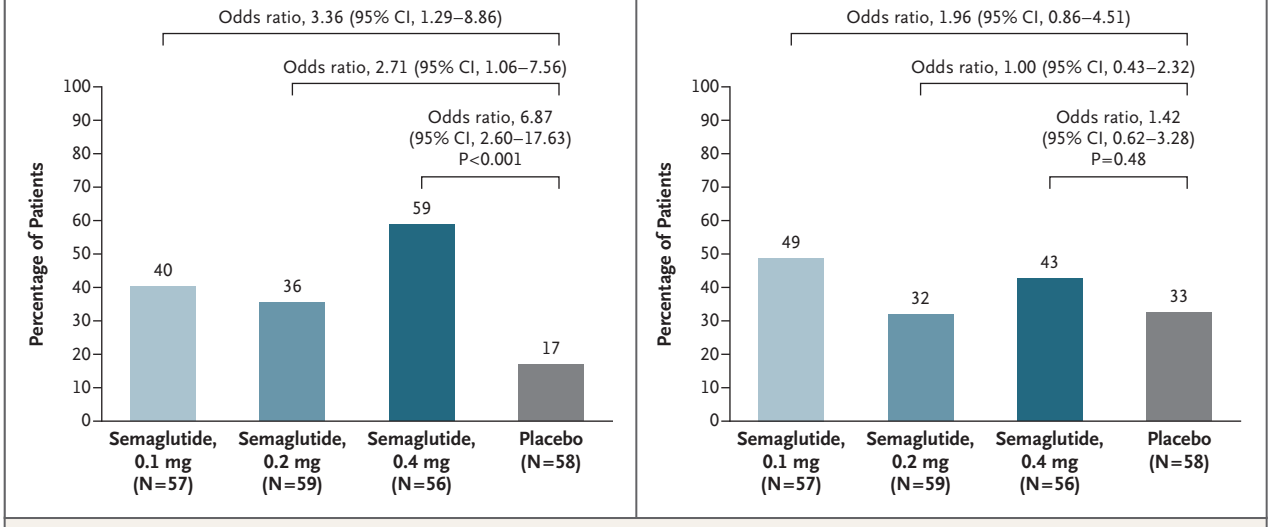

Figure 1. Primary and Secondary Confirmatory End Points.

Panel A shows the observed percentages of patients with stage F2 or F3 fibrosis in whom resolution of nonalcoholic steatohepatitis (NASH) was achieved by week 72 with no worsening of liver fibrosis (with worsening defined as an increase of one stage or more). Resolution was defined by the NASH Clinical Research Network as no more than mild residual inflammatory cells [score of 0 or 1] and no hepatocyte ballooning [score of 0]). Panel B shows the observed percentages of patients with stage F2 or F3 fibrosis who had an improvement of at least one fibrosis stage by week 72 with no worsening of NASH (with worsening defined as an increase of ≥1 point in either the lobular inflammation score or the hepatocyte ballooning score according to the NASH Clinical Research Network criteria). Data were analyzed with the use of a Cochran–Mantel–Haenszel test stratified according to baseline diabetes status and baseline fibrosis stage. Data from the in-trial observation period (from randomization until the last study-related procedure) were included, and missing outcome data were imputed as nonresponse.

observed in 71% of the patients in the semaglutide 0.1-mg group, in 80% in the 0.2-mg group, and in 83% in the 0.4-mg group, as compared with 44% in the placebo group. Changes in the individual activity scores for the components of nonalcoholic fatty liver disease are shown in Figure S7 and Tables S5 and S6.

Dose-dependent reductions in liver-enzyme levels and exploratory biomarker levels were observed with semaglutide (Table 2, Fig. 2, and Table S7). Changes in the score on the serum enhanced liver fibrosis test and in liver stiffness are shown in Table S7 and Figure S9.

Treatment with semaglutide resulted in dose-dependent reductions in body weight (Table 2 and Table S7). The mean percent changes in body weight were –5% in the semaglutide 0.1-mg group, –9% in the 0.2-mg group, –13% in the 0.4-mg group, and –1% in the placebo group. Weight loss in the semaglutide groups continued until approximately weeks 28 to 44 and was sustained thereafter (Fig. S10). Dose-dependent reductions in glycated hemoglobin levels were observed in the semaglutide groups in patients with or without type 2 diabetes (Table 2, Table S7, and Figs. S10 and S11). Changes in lipid levels are summarized in Table 2 and Table S7.

SAFETY

Gastrointestinal disorders were the most common adverse events reported. The percentages of patients with nausea, constipation, decreased appetite, vomiting, and abdominal pain were higher in the semaglutide 0.4-mg group than in the placebo group (nausea, 42% vs. 11%; constipation, 22% vs. 12%; decreased appetite, 22% vs. 5%; vomiting, 15% vs. 2%; and abdominal pain, 7% vs. 4%) (Table 3 and Tables S8 and S9). The timing of the onset of nausea is shown in Figure S12.

The percentage of patients who discontinued treatment because of adverse events was 7% with semaglutide (all doses) and 5% with placebo (Table 3). Gastrointestinal disorders were the

Table 2. Changes between Baseline and Week 72 in Selected Supportive Secondary End Points.*

End Point	Semaglutide 0.1-mg Group (N = 80)	Semaglutide 0.2-mg Group (N = 78)	Semaglutide 0.4-mg Group (N = 82)	Placebo Group (N = 80)
Ratio of value at wk 72 to value at baseline				
Alanine aminotransferase	0.63	0.58	0.42	0.81
Aspartate aminotransferase	0.70	0.65	0.52	0.84
Caspase-cleaved cytokeratin-18 fragment M30†	0.55	0.50	0.47	0.78
Caspase-cleaved cytokeratin-18 fragment M65†	0.53	0.52	0.42	0.71
Total cholesterol	0.97	1.00	0.93	0.94
Triglycerides	0.88	0.90	0.73	0.97
Liver stiffness, as assessed by FibroScan‡	0.76	0.71	0.72	1.02
Change from baseline to wk 72				
Enhanced liver fibrosis test score	−0.34	−0.39	−0.56	0.01
Body weight — %	−4.84	−8.91	−12.51	−0.61
Glycated hemoglobin level among patients with type 2 diabetes — percentage points§	−0.63	−1.07	−1.15	−0.01

* Data are from all the patients during the in-trial observation period (from randomization until the last study-related procedure). A lower ratio of the value at week 72 to the value at baseline indicates a larger reduction.
† Higher levels of cytokeratin-18 fragments are a biomarker of hepatocyte apoptosis.
‡ This assessment was performed only at sites at which FibroScan equipment was available. Changes in liver steatosis were assessed in 161 patients, and changes in liver stiffness were assessed in 212 patients.
§ These values were based on the number of patients with type 2 diabetes in each group (49, 51, 49, and 50 patients in the 0.1-mg, 0.2-mg, 0.4-mg, and placebo groups, respectively).

most common reasons for discontinuation among patients who received semaglutide (4% of the patients); no patients in the placebo group discontinued because of gastrointestinal disorders. The time to discontinuation of semaglutide is shown in Figure S13. Serious adverse events were reported in a higher percentage of patients in the semaglutide groups (15 to 19% across the dose groups) than in the placebo group (10%), but there was no apparent dose-dependent relationship (Table S10).

The incidence of hepatic events was similar across all treatment groups. Gallbladder-related disorders occurred in a higher percentage of patients in the semaglutide groups than in the placebo group (6% in the 0.1-mg group, 5% in the 0.2-mg group, 7% in the 0.4-mg group, and 2% in the placebo group) (Table S11 and Fig. S14). No cases of acute pancreatitis were reported. Severe hypoglycemic episodes were rare, occurring in 2 or fewer patients per group (Table S12).

Neoplasms were not adjudicated. Malignant neoplasms were reported in 3 patients (1%) who received semaglutide (1 with breast cancer in the 0.1-mg group; 1 each with endometrial adenocarcinoma and peripheral T-cell lymphoma in the 0.2-mg group) (Table 3 and Table S13) and in no patients who received placebo. Overall, benign, malignant, and unspecified neoplasms (including cysts and polyps) were reported in 15% of the patients in the semaglutide groups (10 [12%] in the 0.1-mg group, 11 [14%] in the 0.2-mg group, and 14 [17%] in the 0.4-mg group) and in 8% of those in the placebo group (6 patients); no pattern of occurrence in specific organs was observed (Table S14). The most common neoplasms (occurring in >2% of the patients in any treatment group) were a polyp in the large intestine (1 patient in the semaglutide 0.1-mg group, 4 in the 0.2-mg group, and 3 in the 0.4-mg group) and a renal cyst (3 patients in the 0.1-mg group, 1 in the 0.2-mg group, and 1 in the placebo group) (Table 3 and Table S14). Three patients had cardiovascular events confirmed by the event-adjudication committee: two events in 1 patient in the semaglutide 0.2-mg group, one event in 1 patient in the semaglutide 0.4-mg group, and a fatal event (sudden cardiac death)

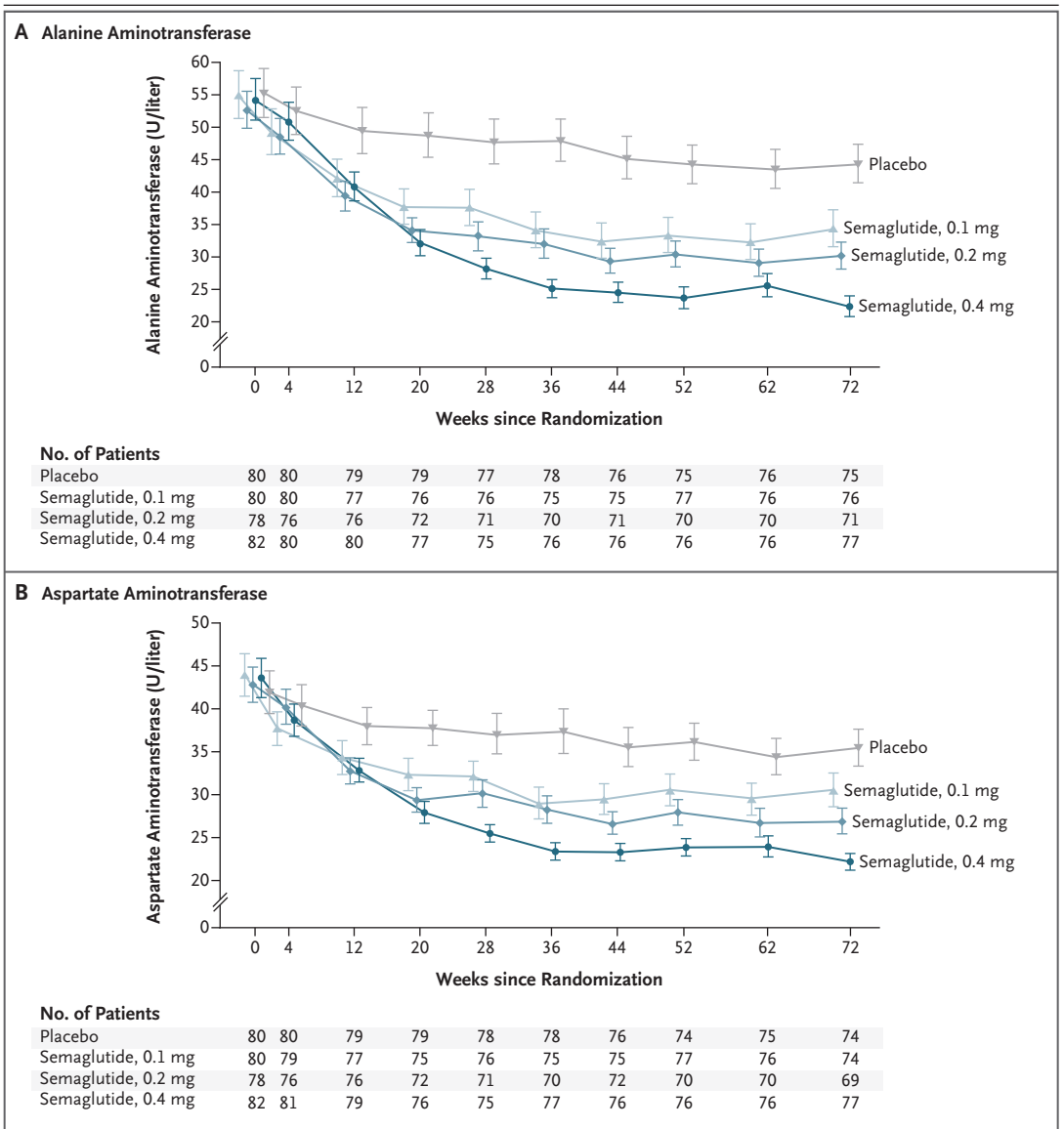

Figure 2. Mean Liver-Enzyme Levels over Time.

Shown are observed mean alanine aminotransferase and aspartate aminotransferase values from baseline through week 72. Data from the in-trial observation period (from randomization until the last study-related procedure) were included.

in 1 patient in the semaglutide 0.2-mg group (Table S15).

Semaglutide was associated with increases from baseline to week 72 in amylase and lipase levels that were greater than those in the placebo group (Table S16). The mean estimated glomerular filtration rate declined slightly across all treatment groups, including the placebo group, from baseline to week 72 (Fig. S15). No safety concerns were noted with respect to other biochemical or hematologic variables, including calcitonin levels and clinical assessments. By the end of the trial, there was no clinically relevant difference in the pulse rate between the semaglutide and placebo groups (difference of 1 to 2 beats per minute) (Table S16).

DISCUSSION

Among patients with stage F2 or F3 fibrosis, once-daily subcutaneous treatment with semaglutide at a dose of 0.4 mg was superior to pla-

Table 3. Selected Adverse Events.*

Event	Semaglutide 0.1-mg Group (N=80)	Semaglutide 0.2-mg Group (N=78)	Semaglutide 0.4-mg Group (N=81)	Placebo Group (N=80)
	number of patients (percent)			
Any adverse event	72 (90)	76 (97)	76 (94)	67 (84)
Adverse events from gastrointestinal disorders system organ class	51 (64)	60 (77)	55 (68)	36 (45)
Adverse events from any system organ class, according to preferred term†				
Nausea	24 (30)	29 (37)	34 (42)	9 (11)
Constipation	13 (16)	17 (22)	18 (22)	10 (12)
Decreased appetite	16 (20)	18 (23)	18 (22)	4 (5)
Diarrhea	23 (29)	22 (28)	16 (20)	11 (14)
Vomiting	14 (18)	17 (22)	12 (15)	2 (2)
Back pain	7 (9)	5 (6)	10 (12)	7 (9)
Headache	7 (9)	10 (13)	10 (12)	8 (10)
Nasopharyngitis	11 (14)	15 (19)	10 (12)	12 (15)
Arthralgia	0	4 (5)	9 (11)	7 (9)
Fatigue	7 (9)	8 (10)	7 (9)	7 (9)
Abdominal pain	9 (11)	8 (10)	6 (7)	3 (4)
Abdominal distension	1 (1)	8 (10)	4 (5)	4 (5)
Dyspepsia	4 (5)	9 (12)	4 (5)	5 (6)
Adverse events that resulted in premature discontinuation of treatment				
All adverse events	3 (4)	10 (13)	4 (5)	4 (5)
Gastrointestinal disorders	1 (1)	6 (8)	2 (2)	0
Serious adverse events				
Any serious adverse event	12 (15)	15 (19)	12 (15)	8 (10)
Gastrointestinal disorders	2 (2)	2 (3)	4 (5)	0
Musculoskeletal and connective-tissue disorders	0	1 (1)	3 (4)	1 (1)
Infections and infestations	2 (2)	2 (3)	2 (2)	1 (1)
Neoplasms, including benign, malignant, and unspecified	0	4 (5)	1 (1)	0
Nervous-system disorders	0	3 (4)	1 (1)	0
Metabolism and nutrition disorders	2 (2)	1 (1)	0	1 (1)
Neoplasms‡	10 (12)	11 (14)	14 (17)	6 (8)
Malignant neoplasms	1 (1)	2 (3)	0	0
Polyp in large intestine§	1 (1)	4 (5)	3 (4)	0
Renal cyst§	3 (4)	1 (1)	0	1 (1)
Fatal events	0	1 (1)¶	0	0

* All adverse events occurred during the on-treatment observation period unless otherwise specified. Data are reported for all the patients who received at least one dose of semaglutide or placebo. One patient in the 0.4-mg group underwent randomization but did not receive any study treatment and was therefore excluded from the safety analysis. Additional information on adverse events is provided in Tables S8 through S14.

† Adverse events with an incidence of at least 10% in any treatment group are shown.

‡ These events were identified with the use of a prespecified search that consisted of multiple standardized *Medical Dictionary for Regulatory Activities* (MedDRA) queries and preferred terms from the neoplasm system organ class.

§ These were the most common events (>2% of patients in any treatment group) identified in the neoplasms MedDRA search.

¶ One patient in the semaglutide 0.2-mg group died during the trial (confirmed by the external adjudication committee as sudden cardiac death). The patient had had type 2 diabetes and established cardiovascular disease, and the event was considered as unlikely to be related to semaglutide by both the investigator and the sponsor.

cebo with respect to resolution of NASH without worsening of fibrosis after 72 weeks of treatment, with 59% of the patients in the 0.4-mg group having a response, as compared with 17% of those in the placebo group (odds ratio, 6.87; 95% CI, 2.60 to 17.63; P<0.001). However, semaglutide did not show a significant between-group difference with respect to an improvement of at least one fibrosis stage without worsening of NASH, which occurred in 43% of the patients in the semaglutide 0.4-mg group as compared with 33% in the placebo group (odds ratio, 1.42; 95% CI, 0.62 to 3.28; P=0.48).

The fact that the percentage of patients who had an improvement in fibrosis stage was not significantly higher with semaglutide than with placebo — despite a greater benefit with respect to NASH resolution and dose-dependent weight loss — was unexpected, given that previous studies have suggested that resolution of NASH and improvements in activity scores for the components of nonalcoholic fatty liver disease are associated with regression of fibrosis.[22,23] However, the temporal association among NASH resolution, weight loss, and improvement in fibrosis stage is not fully understood. It is possible that the current trial was not of sufficient duration for improvements in fibrosis stage to become apparent, especially since most of the patients had advanced fibrosis. Moreover, outcomes such as the score on the enhanced liver fibrosis test and the degree of liver stiffness are continuous variables, and therefore, they may show changes that are not evident from categorical liver-biopsy evaluation. A lack of adequate statistical power for this secondary end point may also have contributed to the unanticipated results.

Although the percentage of patients in the placebo group who had an improvement in fibrosis stage in this trial was similar to the percentage of patients in the placebo group of the PIVENS (Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis) trial who had an improvement in fibrosis stage (31%),[24] it was greater than that reported in several other trials.[10,25,26] No single explanation for this response among patients with NASH who received placebo has been identified.

The potential effectiveness of GLP-1 receptor agonists in the treatment of NASH was previously explored in the 48-week LEAN (Liraglutide Safety and Efficacy in Patients with Non-alcoholic Steatohepatitis) trial. In that trial, NASH resolution (without worsening of fibrosis) was reported in 39% of the patients who had received once-daily liraglutide at a dose of 1.8 mg as compared with 9% of the patients who had received placebo; in addition, liraglutide treatment was associated with the prevention of worsening fibrosis.[10] In the current trial, worsening of fibrosis occurred in 5% of the patients in the semaglutide 0.4-mg group and in 19% of the patients in the placebo group.

Given the lack of hepatic GLP-1 receptor expression,[27] the potential mechanism of action of GLP-1 receptor agonists in NASH may relate to indirect beneficial effects on weight and insulin resistance, as well as reductions in metabolic dysfunction, lipotoxic effects, and inflammation.[4,5,27-29] Preclinical NASH studies have suggested that semaglutide reduces liver inflammation through mechanisms that are, in part, independent of weight loss[27]; in the current trial, we observed reductions in the levels of biomarkers of inflammation and in histologically assessed lobular inflammation.

The safety profile of subcutaneous semaglutide was consistent with that observed in patients with type 2 diabetes in other trials and with the known effects of GLP-1 receptor agonists.[14-17,30] Gastrointestinal disorders, including nausea, constipation, vomiting, and abdominal pain, were the most commonly reported adverse events among the patients who received semaglutide. These events occurred primarily in the first 20 weeks of the trial, during the period of dose escalation of semaglutide. Gallbladder-related adverse events were more common with semaglutide than with placebo, and increases in amylase and lipase levels were greater with semaglutide than with placebo.

Overall, the incidence of observed events of benign, malignant, and unspecified neoplasms (none of which were adjudicated) was numerically higher in the semaglutide groups than in the placebo group, with no pattern of occurrence in specific organs observed. Malignant neoplasms occurred in 3 patients who received semaglutide and in no patients who received placebo. In a recent meta-analysis of data from 55,921 patients, GLP-1 receptor agonists, including sema-

glutide, were not associated with an increased risk of malignant neoplasms.[31] Cardiovascular events, which were adjudicated by an external adjudication committee, occurred in 3 patients (four events), all in the semaglutide groups. However, the trial was not powered to evaluate cardiovascular outcomes, and no conclusions can be drawn because of the small number of events.

Our phase 2 trial involved patients with or without type 2 diabetes, who had biopsy-confirmed NASH at baseline after central evaluation by two pathologists and had week-72 biopsies that were evaluated by the same two pathologists. Although the pathologists were not always in agreement on all variables, the interreader variability was similar to that previously reported,[19,22] and consensus was ultimately reached in all cases. Possible limitations of our trial include intraobserver variability, as well as a lack of long-term clinical outcomes.

Among patients with biopsy-confirmed NASH and fibrosis, a significantly higher percentage of patients had NASH resolution with once-daily semaglutide than with placebo. The trial did not show a significant between-group difference in the percentage of patients with an improvement in fibrosis stage.

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APPENDIX

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