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Journal of Diabetes, Metabolic Disorders & Control

Research Article
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Effect of dulaglutide treatment in aged subjects with type 2 diabetes mellitus and fatty liver disease

Summary

Fatty liver (NAFL) often comes in association with type 2 diabetes mellitus (T2DM). NAFL disease (NAFLD) evolution towards non-alcoholic steatohepatitis (NASH), liver cirrhosis, and cancer-cirrhosis is a further challenge for diabetologists and further increases the cardiovascular risk (CV-R) in people with T2DM and especially in aged ones. Conversely, various reports in the literature suggest glucagon-like-peptide-1 receptor agonists (GLP-1RAs) improve both liver function and CV-R. The least studied GLP-1RA is dulaglutide. Based on such considerations, we set up an open-label, 24-month duration, single-blind, prospective, multicenter randomized case-control study involving 500 patients over sixty years of age with T2DM to assess the effects of dulaglutide 1.5 mg/week on validated, user-friendly, indirect markers of liver steatosis and fibrosis frequently used in an outpatient setting, including ultrasound-based Fatty Liver Index (FLI), FIB-4 and NAFLD Fibrosis Score (NFS) without resorting to invasive and costly tests, including biopsies or nuclear magnetic resonance. We could thus show that dulaglutide treatment associates with decreased FLI levels in a significant percentage of patients and, by a lesser degree, with decreased FIB-4 scores. Such results encourage dulaglutide utilization in people with T2DM and liver steatosis to reduce the rate of progression toward cirrhosis/cancer-cirrhosis and CV-R.

Keywords: diabetes, dulaglutide, NASH, NAFLD, FLI, FIB-4, NFS

Volume 10 Issue 1 - 2023

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Received: May 14, 2023 | **Published:** May 29, 2023

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common hepatopathy worldwide. It represents a large disease spectrum ranging from mere liver steatosis (non-alcoholic fatty liver) to non-alcoholic steatohepatitis (NASH), i.e., the previous one complicated by inflammatory cell injury, which, due to its frequent progression to cirrhosis and hepatocellular carcinoma,[1] is expected to become the leading cause of liver transplantation soon.[2] NAFLD is strongly associated with several components of the metabolic syndrome[3] and is present in many patients with Type 2 diabetes mellitus (T2DM).[4] Peripheral insulin resistance plays a major role in the pathogenesis of NAFLD and its sequelae by causing ectopic fat accumulation through increased lipolysis and delivery of free fatty acids to the liver and, if intense and consistent, can generate the extensive liver inflammatory and fibrotic changes characterizing NASH.[5] Glucagon-like peptide-1 receptor agonists (GLP-1RAs) benefit liver biomarkers in people with NAFLD/NASH. In animals, GLP-1RAs reduced weight, liver mass and lipid content, plasma alanine aminotransferase (ALT) levels, and fibrosis[6–8] while improving hepatic insulin sensitivity.[9] Liraglutide improved hepatic steatosis in people with T2DM[10] and reduced NASH-associated damage in human and animal models through modulated inflammatory signaling pathways.[11] Exendin-4, i.e., another GLP-1RA, markedly reduced hepatic triglyceride accumulation by directly activating fatty hepatocyte GLP-1 receptors[12] in the absence of insulin. Thus, independently of weight loss, GLP-1RAs greatly benefit people with T2DM and NAFLD. Weekly 1.5 mg subcutaneous dulaglutide for 24 weeks significantly reduced serum AST, ALT, and gGT levels in a pooled series of 290 AWARD clinical trial patients with T2DM and NAFLD/NASH compared to placebo.[13] These results look promising but are not confirmed by studies on dulaglutide. A recent meta-analysis included exenatide and liraglutide (either alone or combined with other oral hypoglycemic agents or insulin) but ignored dulaglutide, despite the latter being largely used for years at the time of publication.[14]

NASH and NAFLD are closely linked to metabolic syndrome and a severe risk for cardiovascular events,[15] including stroke,[16] especially in the over-sixties. Such consideration suggests severe liver steatosis and fibrosis monitoring in as many obese people with T2DM as possible. However, liver biopsy, i.e., the best diagnostic tool for steatosis and fibrosis,[14] is not feasible on a large scale for its complex, costly, and invasive procedure, which makes it seldom accepted to patients.[17] Ultrasonography (US) and fibroscan can also help in NASH/NAFLD monitoring, despite doubts on their ability to

quantify liver damage.[18] Luckily, some inexpensive outpatient liver damage biomarkers were recently validated, exploiting user-friendly anthropometric and laboratory parameters, including Fatty Liver Index (FLI), FIB-4 score, and NAFLD Fibrosis Score (NFS).[19,20,21–25] Based on the abovementioned considerations, we designed the present 24-week prospective, single-blind, usual care, randomized, case-control clinical trial to test dulaglutide's ability to improve steatosis and fibrosis in subjects with NASH/NAFLD and T2DM and subordinately, to confirm its positive effects on serum AST, ALT, and gGT levels.

Methods

Participants and setting

The study aimed to evaluate the effects of once-weekly subcutaneous dulaglutide 1.5 mg administration on liver fat content and fibrosis. The study was designed and conducted in agreement with the CONSORT guidelines in ten diabetes care outpatient units (DCOUs) operating under the umbrella of a single entity joint with the Campania University "Luigi Vanvitelli", Naples, Italy called Nefrocenter Research and using the same centralized laboratory and the same database to archive patient data. The clinical trial protocol was approved by the Institutional Review Board and Ethics Committee of Vanvitelli University as the reference center for all the other joint units (protocol n. 1226/2019 of 07/06/2019). All DCOUs were certified for successful participation of all involved Health Care Providers (HCPs) in the structured training on all trial procedures according to the Quality of Care Improvement Program from the Associazione Medici Diabetologi (AMD; www.aemmmedi.it).

All participants provided informed written consent to the study.

Subjects

Five hundred patients with T2DM met the inclusion criteria, signed the informed consent, and entered the present study after accepting sequential, competitive enrollment. The protocol is graphically summarized as a flow chart in Figure 1.

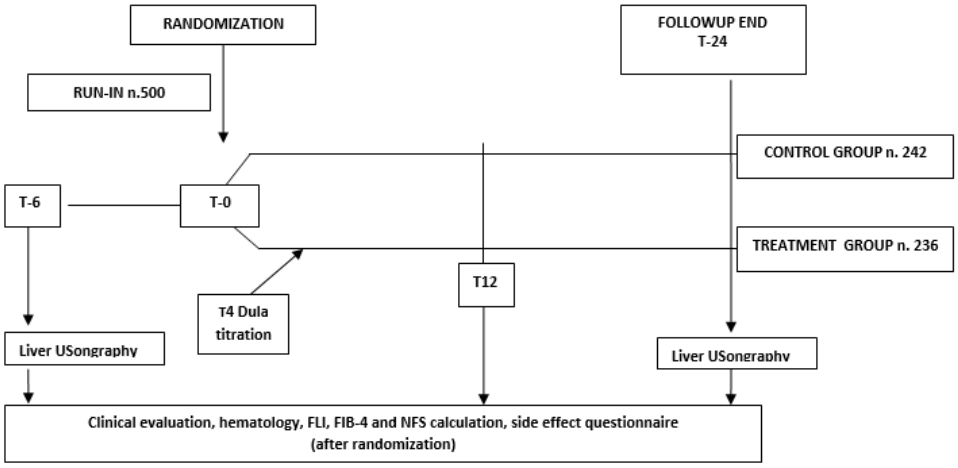

Figure 1 Flow-chart of the study.

Type of study

Two-arm, open-label, single blind, prospective, multicentre randomized case-control study.

Competitive enrollment up to targeted participant number.

The two arms consisted of the **Treatment Group (TG)**, administered dulaglutide 1.5 mg weekly as an add-on to standard therapy, and the **Control Group (CG)** receiving standard therapy.

Eligibility criteria

Patients were included when meeting the following criteria:

I. >65 years of age; baseline US-detected liver fatty content (LFC) ≥6.0%;[26]

II. AST and/or ALT within 5 times the upper limit of normal (ULN);[26]

III. T2DM on poor control (HbA1c >53 mmol/mol [>7.0%]) with standalone or associated dipeptidyl peptidase 4 (DPP-4) inhibitors, sulfonylureas, insulin and metformin (which, despite being of help on fatty liver content, was known not to affect fibrosis, and, as the most frequently used antihyperglycemic agent, was equally represented in both treatment arms.[27]

Exclusion criteria were

I. Severely ill-controlled diabetes (HbA1c >86 mmol/mol [>10.0%]);

II. Alcohol consumption >14 units per week for women and >21 units per week for men;

III. Severe hepatic impairment of any cause (AST or ALT >5 times the ULN)

IV. Evidence of other forms of liver disease, including hepatitis B, hepatitis C and autoimmune hepatitis; use of drugs known to cause hepatic steatosis;

V. Treatment with glucose-lowering drugs other than metformin known to influence liver fat content, including thiazolidinediones, α-glucosidase inhibitors, sodium–glucose cotransporter 2 (SGLT2) inhibitors, and any GLP-1RA agonists for the previous 3 months;

VI. Treatment with vitamin E for the previous 3 months;

VII. Contraindications to dulaglutide use (history of acute or chronic pancreatitis, unexplained abdominal pain, pancreatic cancer or personal or family history of current, or previous thyroid malignancy).

Citation: Gentile S, Strollo F, Guarino G et al. Effect of dulaglutide treatment in aged subjects with type 2 diabetes mellitus and fatty liver disease. *J Diab Metab Disorder.* 2023;10(1):56–64. DOI: 10.15406/jdmdc.2023.10.00253

Baseline assessment

All participants underwent baseline assessment before randomization, including anthropometry, detailed medical history, and physical examination. Alcohol use assessment was documented using the AUDIT questionnaire.[28] Blood tests included fasting plasma glucose and HbA1c, liver function tests (total bilirubin, albumin, ALT, AST, gGT, alkaline phosphatase [ALP]), kidney function tests (serum urea, creatinine, uric acid, spot urine protein/creatinine ratio), electrolytes (sodium, potassium), lipid profile (triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol), complete blood counts (including hemoglobin and total red blood cell, leucocyte and platelet count), hepatitis B surface antigen, and anti-hepatitis C and HIV I and II antibodies. Baseline assessment also included liver US with the FLI, FIB-4 index, and NFS calculation.

Randomization and allocation concealment

Participants were randomized into either the dulaglutide or the control group based on a predefined computer-generated number with a 1:1 open-label allocation concealed through serially numbered, opaque envelopes. Investigators involved in imaging data analysis were blinded to participants' information and allocation sequence. Although aware of the treatment group, consultant diabetologists were blinded to the results until the final data analysis. The drug used was dulaglutide (Trulicity©; Eli Lilly, USA).

Dulaglutide titration: the starting dulaglutide dose of 0.75 mg / week was maintained for 4 weeks. Then, in the absence of any side effects or intolerance symptoms, it was increased to 1.5 mg/week throughout follow-up. The injection of dulaglutide was performed following the correct injection techniques by constantly rotating the injection sites and injecting the room-temperature drug according to the recommendations on correct AMD-OSDI injection techniques.[29]

Study visits

After the baseline visit, participants meeting all inclusion/exclusion criteria were randomized to receive 24 weeks of either the standard treatment (control group, CG) or dulaglutide as an add-on to it (treatment group, TG). All received proper diet and lifestyle training through educational refreshers and information concerning potential adverse drug reactions with explicit requests to document all symptoms experienced during the study period, whether or not related to the drug. In both groups, adjustment of diabetes treatment was carried out based on self-monitored blood glucose at weeks 6, 12, and 18. Glycaemic equipoise between groups was maintained as high as possible by adjusting glucose-lowering drugs (metformin, DPP-4 inhibitors, sulfonylureas, and insulin) in the control group to minimize the effects of different degrees of hyperglycemia on liver fat in the two groups according to prespecified glucose targets in agreement with the ADA guidelines 2018.[30] All participants were instructed to restrict fast-absorbed carbohydrates (avoid fruit juices and simple sugars and reduce rice utilization) and fat intake (reduce butter and cheese). All participants were advised to exercise (brisk walk) for at least 30 min a day for at least 4 days a week. Participants received uniform lifestyle modification instructions at baseline and week 12, in agreement with the standards of diabetes management, and returned to the outpatient DCOU for follow-up visits at weeks 6, 12, 18, and 24.[31]

Outcomes

The primary outcome measure in subjects with US signs of fatty liver was the differences in advanced fibrosis markers, i.e., FIB-4 index, FLI and NFS, between week 24 (T24) and baseline (T0). Secondary outcome measures included the differences in serum AST, ALT, and gGT levels observed during the same period.

FLI, FIB-4 score, NAFLD fibrosis score (NFS) protocol

FLI

According to a previous study,[19] a validated Fatty Liver Index (**FLI**) between 0 and 100 (multiplying the predicted probabilities per 100) was derived from the following formula:

$$FLI = (e^{0.953*\log e\ (triglycerides) + 0.139*BMI + 0.718*\log e\ (\gamma GT) + 0.053*waist\ circumference - 15.745)} / (1 + e^{0.953*\log e\ (triglycerides) + 0.139*BMI + 0.718*\log e\ (\gamma GT) + 0.053*waist\ circumference - 15.745)} x\ 100$$

A FLI ≥ 60 indicates hepatic steatosis (SP = 86%; LR+ = 4.3) and < 30 rules it out (SN = 87%; LR- = 0.2), where SN = sensitivity; SP = specificity; LR+ = positive likelihood ratio; LR- = negative likelihood ratio. A score between 31 to 59 is considered inconclusive.

FIB-4 and NFS

According to previous studies,[22,23] the **FIB-4** and the **NFS** (NAFLD Fibrosis Score) are two easy to handle, validated, non-invasive surrogate markers of fibrosis based on routine laboratory tests and anthropometric and anamnestic data, as described by the following formulas:

$$\mathbf{FIB-4}:\ (Age\ x\ AST)\ /\ (Platelets\ x\sqrt{ALT})$$

where a score <2.0 = lower risk for advanced fibrosis (F3/F4) in subjects over 65 years of age and a score > 2.67 = high risk for advanced fibrosis for all ages, where F0 = no fibrosis; F1 = portal fibrosis without intralobular septa; F2 = portal fibrosis with few intralobular septa; F3 = numerous septa without cirrhosis; F4 = cirrhosis.

- The correlation between FIB-4, Fibrotest and elastography with histological fibrosis is excellent)[32]

$FIB-4\ evaluation$

$In\ NASH\ (non-alcoholic\ steatohepatitis):$

$-\ Fibrosis\ F0-F1\ (FIB-4\ <\ 1.30)$

$-\ Cirrhosis\ F3-F4\ (FIB-4\ >2.67)$

$In\ HCV\ with\ or\ without\ HIV:$

$-\ Fibrosis\ F0-F1\ (FIB-4\ <\ 1.45),$

$-\ Cirrhosis\ F3-F4\ (FIB-4\ >\ 3.25)$

NAFLD Fibrosis Score (NFS)

NFS can be calculated using the following formula[33]

$$-1.675 + (0.037\ x\ age) + (0.094\ x\ BMI) + (1.13\ if\ IFG\ or\ overt\ diabetes) - (0.013\ x\ platelets) - (0.66\ x\ albumin)$$

where IFG = Impaired Farting Glucose

Interpretation

I. a score < 0.12 = lower risk for advanced fibrosis (F3/F4) in subjects over 65 years of age

II. a score > 0.676 = high risk for advanced fibrosis (F3/F4) for all ages.

The cost for their calculation is negligible and the result is immediate. Both scores were developed and validated for the identification of patients with NAFLD with a high probability of having bridging fibrosis (F3) or cirrhosis (F4). A FIB-4 score > 2.67, and an NFS > 0.676 identify subjects with a high probability of advanced liver fibrosis (F3/F4).[20,23–25]

Ultrasonography

Liver ultrasonography was performed by last generation Esaote MyLab™X75 equipped with SI2C41 convex ultrasound probe (Esaote S.p.A., Genoa, Italy) following a validated procedure having a sensitivity of 100% and a specificity of 90% for the diagnosis of liver fat, when there was □20% of fat, compared with liver biopsy.[18] However, as precise liver fat quantification still lacks validation on large numbers,[19,31] we asked the same operator to perform US only to get fatty liver checked at enrolment, and liver fat content was calculated through FLI, with an inter-assay variability <10%.

Biochemical measurements at follow-up

History of prescribed medications and their adverse reactions were noted, and anthropometry, physical examination results, and biochemical measurements were recorded for each participant at baseline and at week 24. Venous blood samples were taken in the morning after participants had fasted overnight for 12 h, and the samples were analyzed on the same day at the center laboratory of the hospital for the following variables: fasting plasma glucose (FPG); HbA1c; liver function test (total bilirubin, albumin, AST, ALT, gGT, alkaline phosphatase); kidney function test (urea, creatinine, uric acid, urine protein/creatinine ratio), lipid profile (triglycerides, total cholesterol HDL, LDL); complete blood counts (hemoglobin, total leucocyte count, platelet count); and TSH. In particular, we assumed as normal values between 5 and 55 U/L for AST, 8-48 U/L for ALT, 5-40 for gGT, and 40-174 IU/L for alkaline phosphatase.

Sample size calculation

We assumed a 5.0% absolute difference in LFC between dulaglutide and control groups would be the minimally appreciable and clinically relevant difference. Based on the results of previous similar clinical studies involving ezetimibe, sitagliptin, and empagliflozin,[26,35,36] we expected the dulaglutide group to have an absolute liver fat reduction of >5.0% compared with baseline and the control group to have <2.0% reduction in liver fat compared with the baseline, and a dropout of <10%. With this premise, the sample size per group worked out as 98 per group to achieve a power of at least 90% with a β of 0, 01. Therefore, we planned to randomize 250 participants per group to ensure adequate study power even with dropouts.

Statistical procedure

Categorical data were presented as n (%). Continuous descriptive data were examined for normality, and normally distributed data are presented as mean±SD. Skewed data are presented as median (IQR). The $\chi 2$ test or Fisher's exact test were used for categorical variables, and the independent sample Student's t-test or Wilcoxon–Mann–Whitney U test were used to compare differences between continuous variables. The Pearson correlation coefficients (r) were determined by linear regression analysis. Additional analyses of primary and secondary outcomes within treatment groups were performed by using two-tailed independent sample t-tests, paired t-tests, or nonparametric tests when indicated. Univariate logistic regression was performed to screen for potential covariates associated with liver fat improvement. Covariates with $p < 0.01$ were entered into the multivariable model using the forward condition method. Multivariate linear regression analysis was conducted to reveal the estimated effect of dulaglutide on liver fat. Statistical analyses were performed by a biostatistician (MKS). All the statistical analyses were performed using the SAS System version 9.4 (SAS Institute, Cary, NC, USA).

Results

Twenty-two enrolled participants dropped out for treatment change, metabolic failure, or personal reasons. Therefore, at the end, of the original 250-subject cohorts per group, 242 and 236 participants completed their follow-up until the 24th month (T24) in the CG and TG, respectively. Table 1 displays general parameters, clearly showing superimposable values between groups. In particular, a typical NASH FLI was observed at high rates in both the CG and the TG (94.73% vs. 95.91%, respectively). A severe FIB-4 score (i.e., F3-F4) was present in 84.21% vs. 85.71, respectively, a medium one (i.e., F2) in 4.85% vs. 4.08%, respectively, and a normal/light one (F0-F1) in 10.93% vs., 10.20%, respectively. A similar trend was found at the NFS level for high and low fibrosis risk s(i.e., 90.28% vs. 91.02%, and 5.66% vs. 5.30% respectively), with inconclusive results only observed at nominal rates (5,66% vs. 3.26%, respectively). No participants had severe adverse effects, needed hospitalization, or died due to accidental causes or treatment. 12,7% vs. 16,5% of subjects had symptomatic non-severe hypoglycemia in the CG and TG, respectively. Notably, most cases in the TG were reported within the first three months of follow-up. Severe hypoglycemia requiring caregiver assistance was 17 and 21, respectively.

Table 1 General features of the two groups

	Control group (n. 242)	Treatment Group (n. 236)	p
Age (years)	72.6 ± 6.5	71.9 ± 5.8	ns
Sex *(M/F)*	98/149	101/144	ns
Diabetes duration *(years)*	7.4 ± 3.6	7.7 ± 4.6	ns
BMI *(kg/m2)*	35.5±4.4	36.4±5.2	ns
HbA1c *(%)*	8.1±1.7	8.3±1.9	ns
FPG *(mg/dl)*	137.9±16.8	131.5±14.8	ns
PPG *(mg(dl)*	189.5±22.4	191.7±4.3	ns
ASL *(IU/L)*	68.5±6.6	69.6±8.6	ns
ALT *(IU/L)*	65.6±8.3	71.9±9.4	ns
γGT *(IU/L)*	59.4±4.8	61.5±7.2	ns
Alkaline phosphatase *(IU/L)*	189.5±14.4	190.8±14.6	ns
Total cholesterol *(mg/dl)*	189.7±18.6	185.8±19.3	ns
HDL Cholesterol *(mg/dl)*	43.2±4.3	44.1±3.2	ns
Triglyceride *(mg/dl)*	189.7±22.5	190.4±24.8	ns

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Table 1 Continued...

		Control group (n. 242)	Treatment Group (n. 236)	p
LDL Cholesterol *(mg/dl)*		107.5±8.9	193.5±9.9	ns
Platelet count *(n/mL)*		165,000±18,000	160,000±21,000	ns
Albumin *(g/dl)*		3.9±0.4	3.9±0.5	ns
eGFR *(ml/min/1.73m2)*		82.6±7.9	91.4±8.6	ns
FLI < 30	*n. (%)*	8 (2.23)	7 (2.85)	ns
FLI > 60	*n. (%)*	233 (94.73)	226 (95.91)	ns
FLI 31-59	*n. (%)*	5 (2.02	3 (1.22)	
FIB score F0-F1 <1.30	*n. (%)*	27 (10.93)	25 (10.20)	ns
FIB score F3-F4 >2.27	*n. (%)*	207 (84.21)	201 (85.71)	ns
FIB score F2	*n. (%)*	12 (4.85)	10 (4.08)	ns
NSF score <1.12	*n. (%)*	12 (5.66)	13 (5.30)	Ns
NSF score >0.676	*n. (%)*	222 (90.28)	219 (91.02)	Ns
NSF score Unconclusicìve	*n. (%)*	12 (5.66)	8 (3.26)	ns
Hypoglycemic Treatment				
Metfromin *n. (%)*		131 (53.0)	139 (56.7)	ns
Secretagogues *n. (%)*		31 (12.6)	25 (10.2)	ns
DPP-4i *n. (%)*		40 (16.2)	44 (17.9)	ns
Insulin *n. (%)*		45 (18.2)	36 (14.7)	ns
Diabetes Chronic Complications / Comorbidities *(one or more)*				
Cardio-vascular disease *n. (%)*		155 (62.7)	148 (60.4)	ns
Hypertension *n. (%)*		189 (76.5)	176 (71.8)	ns
Renal Disease *n. (%)*		38 (15.3)	40 (16.3)	ns
Retinopathy *n. (%)*		74 (29.9)	82 (33.5)	ns
Neuropathy *n. (%)*		68 (27.5)	59 (24.1)	ns
Foot ulcer/amputation *n. (%)*		5 (2.1)	3 (1.2)	ns
Previous Stroke *n. (%)*		13 (5.3)	20 (8.2)	ns
Hypercholesterolemia *n. (%)*		131 (53.0)	137 (55.9)	ns

Table 2 Treatment side effects in the two groups

	Nausea	Diarrhea	Vomiting	Abdominal pain	Decreased appetite	Indigestion and fatigue
Control group (%)	6	3	4	5	3	4
Treatment group (%)	28	3	7	3	28	7
p	<0.001	n.s.	n.s.	n.s.	<0.001	n.s.

Table 3 Least squares mean differences (T0 - T24, %) in laboratory parameters between groups

	Control group (n. 247) Δ T0T24 [%]	Treatment group (n. 245) Δ T0T24 [%]	confidence interval 95%	p
Body weight	-1.1±0. 01	-4.6±0.4	2.231 - 6.457	<0.01
HbA1c	-7.5±0. 4	-8.2±1.0	0.586 - 1.318	<0.37
FPG	-7.6±0.9	-7.9±0.8	0.637 - 1.031	<0.49
PPG	-6.5±1.8	-6.6±2.2	1.064 - 1.981	<0.42
ASL	-5.5±1.0	-30.6±12.8	2.294 - 10.174	<0.0001
ALT	-4.4±0.8	-27.6±2.1	1.993 - 10.393	<0.0001
γ GT	-2.2±0.4	-22.2±1.1	1.223 - 6.319	<0.0001
Alkaline phospatase (%)	-3.4±0.8	-20.9±1.8	1.398 - 6.771	<0.0001
Total cholesterol *(mg/dl)*	-5.5±1.0	-5.9±1.1	0.678 - 1.912	<0.556
HDL Cholesterol *(mg/dl)*	+1.1±0.4	+0.9±0.4	0.736 - 2.082	<0.048
Triglycerides *(mg/dl)*	-8.5±1.1	-7.8±0.8	0.912 - 2.112	<0.059
LDL Cholesterol *(mg/dl)*	-7.4±0.9	-7.9±1.1	0.916 - 2.128	<0.045
Platelet count *(n/mL)*	-0.9±0.1	-0.8±0.1	0.349 - 0.916	<0.582
Albumin *(g/dl)*	+0.2±0.05	+0.2±0.04	0.337 - 0.621	<0.761
eGFR *(ml/min/1.73m2)*	-0.4±0.1	-0.5±0.1	0.399- 0.917	<0.618

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Table 4 Least squares mean differences in FLI, FIB-4, and NSF scores between groups at the end of follow-up. Significance of differences vs baseline: * p<0.001

		Control Group (n. 247)	Treatment Group (n. 245)	95% CI	p
FLI < 30	*n. (%)*	**9 (3.6)**	**110 (45.2)***	**1.128 - 3.236**	**<0.001**
FLI > 60	*n. (%)*	**236 (95.2)**	**112 (45.9)***	**2.712 - 6.573**	**<0.001**
FLI 31-59	*n. (%)*	**3 (1.2)**	**23 (8.9)***	**2.931 - 7.311**	**<0.001**
FIB score F0-F1 <1.30	*n. (%)*	**28 (11.3)**	**96 (39.2)***	**1.793 - 5.881**	**<0.001**
FIB score F3-F4 >2.27	*n. (%)*	**209 (84.6)**	**134 (54.7)***	**1.963 - 5.779**	**<0.001**
FIB score F2	*n. (%)*	**10 (4.1)**	**15 (6.1)**	**1.029 - 2.195**	**ns**
NSF score <1.12	*n. (%)*	**12 (5.1)**	**78 (41.8)***	**1.819 - 5.992**	**<0.001**
NSF score >0.676	*n. (%)*	**224 (90.6)**	**157 (44.1)***	**2.085 - 6.789**	**<0.001**
NSFscore Unconclusicive	*n. (%)*	**10 (4.3)**	**10 (4.1)**	**0.387 - 1.936**	**ns**

As reported in Table 2, gastrointestinal adverse effects were clinically irrelevant and comparable between groups, except for nausea (6% vs. 28%, respectively, p<0.001) and poor appetite (3% vs. 28%, respectively, p<0.001). However, such symptoms caused no drop-outs, and dulaglutide did not associate with altered pancreatic enzymes. Table 3 synthesizes the least squares mean T24-T0 between-group treatment differences (-D %) in laboratory parameters. It clearly shows that AST, ALT, gGT, and ALP slightly decreased in the CG while doing significantly so in the TG. In particular, at the end of the follow-up, participants getting down to normal were il 12% vs. 79% for AST (p<0.001), 9% vs. 80% for ALT (p<0.001), 9% vs. 78% for gGT (p<0.001), and 10% vs. 68% for ALP (p<0.001), respectively. Body weight significantly decreased only in the TG. Conversely, HbA1c, FPG, and PPG showed a similar, significant decrease between groups. Instead, lipids, platelet counts, eGFRs, therapeutic regimes, complication rates, and comorbidities did not change significantly in either group.

Table 4 compares percent variations of FLI, FIB-4 e NFS at the end of follow-up, showing complete behavioral overlap. Indeed, no changes were observed over time in any of the three indexes in the CG, while, in the other group, the percent of subjects with FLI <30, indicative of the absence of hepatic steatosis (HS) and non-diagnostic (FLI score 31-59; 1.2% vs. 8.9%) increased at T24 (p<0.001), while that of subjects with FLI >60, indicative of HS presence, significantly decreased (p<0.001). FIB-4 behaved the same. Indeed, at T24, the CG kept FIB-4 levels similar to baseline, while, in the TG, the percent of participants with F0-F1 significantly increased from 11.3% to 39.2% (p<0.001), and that with F3-F4 decreased from 84.6% to 54.7% (p<0.001), with median levels (F2) keeping virtually at the same rate over time (from 4.1% to 6.1%; p ns). When turning to NSF, no changing trends over time were observed in the CG. Conversely, TG subjects with high fibrosis risk (score >0.676) significantly decreased from 90.6% to 64.1% (p<0.001), thus favoring a shift toward the low risk (score <0.12) through an increased prevalence from 5.1% to 31.8%, p<0.001), in the absence of any changes in non-diagnostic cases (from 4.3% to 4.1%, p ns).

Discussion

There has always been great interest in the extra-glycemic effects of drugs against diabetes among investigators, leading to many papers in the field, most recently dealing with the impressive heart- and kidney-protective role of sodium-glucose co-transporter-2 inhibitors (SGLT-2i).[37] Despite attracting the scientific community for their supposed beneficial effects on fatty Liver, GLP-1RAs have only been investigated in animal models[6,7] and in just a few human cases[8–12] or subanalyses of differently oriented RCTs.[13] In any case, despite having been available since 2014, dulaglutide is the least investigated GLP1-RA for this purpose [38]. Therefore, we decided to analyze dulaglutide's ability to improve circulating AST, ALT, gGT, and ALP levels and signs of liver steatosis or fibrosis in obese people over sixty years of age with T2DM and NAFLD/NASH.

The results obtained through a 24-month treatment with dulaglutide at a weekly dose of 1.5 mg confirmed a significant decrease in AST, ALT, gGT, and ALP over time in a significantly higher percentage compared to controls. In particular, in agreement with previous findings, in the TG, the normalization rate achieved at the end of follow-up was 79% vs. 12% for AST, 80% vs. 9% for per ALT, 78% vs. 9% for gGT, and 68% vs. 10% for ALP compared to the CG (p<0.001 consistently).[12,13] Triglyceride levels only slightly decreased in both groups (-8.9+0.8 vs. -7.8+1.1 mg/dl, respectively; p<0.039), thus partially supporting previous observations of significantly reduced triglyceride stores in steatotic hepatocytes following exendin-4 treatment.[12] In our study, during the follow-up HbA1c, FPG, and PPG declined at almost the same rate in both groups, thanks to treatment intensification in the CG and the addition of dulaglutide in the TG to improve metabolic control similarly to rule out biases eventually influencing results. In greater detail, the percent decay throughout follow-up (D T0-T24)[38] was -8.2+1.0 vs. -7.5+0.4 for HbA1c in the TG compared to the CG (p<0.01). Similarly, it was -7.9+0.8 vs. -7.6+0.9 for FPG and -6.6+2.2 vs. -6.5+1.8 for PPG, respectively (p<0.01 in both cases). As expected, dulaglutide's effect was impressive on body weight, which, indeed, significantly decreased by 4.4+0.8% in the TG (p<0.01) while keeping virtually unchanged in the CG (-1.1+0.015, p ns), thus leading to a marked difference between groups at the end of follow-up (p<0.01), in agreement with previous observations.[39]

At the ultrasound scan, fatty liver content was assessed through FLI in subjects with a >6% hepatic steatosis. Despite being an indirect method, FLI was our choice for evaluating diffuse steatosis, considering that quantitative or semi-quantitative estimates of liver fat content are inaccurate and largely operator-dependent. Indeed, NAFLD and steato-fibrosis evaluation with ultrasound-based techniques has improved much in the last few years. However, despite being widely used and accepted, semi-quantitative, quantitative, elastographic, and contrast-enhanced ultrasound techniques are still debated for clinical and research purposes.[18] Moreover, capturing the typical NASH inflammatory component is still quite tricky.

Our study's concordance between US evaluation and FLI at baseline was as high as 94.73% in the CG and 95,81% in the TG, leaving only 2.02% and 1.22% to non-diagnostic cases, and 2.23% and 2.85% to negative cases, respectively. The end-of-follow-up evaluation showed a significant decrease to 45.9% of steatosis-indicative FLI (p<0.001) in the TG in the absence of substantial changes (final observation: 95.2%) in the CG (p ns). Our results are particularly relevant as they are independent of any glucose control changes and support previous observations in animal and human

studies.[10–14] Liver fibrosis results from extracellular matrix (ECM) protein accumulation in most chronic liver diseases,[40] generating scarring processes which, in end-stages, subvert liver architecture and cause hepatocellular regeneration. In other words, progressing fibrosis leads to initially compensated, then overt cirrhosis, eventually causing liver failure and portal hypertension.[40]

Fibrosis severity is the most relevant predictor of adverse hepatic outcomes in patients with NAFLD[25,41,42] because portal fibrosis and porto-portal bridging typically induce hepatic nodules destroying the original liver structure until cirrhosis comes about: all these elements are difficult to define even though the most updated US technology.[18] Thus, FIB-4 and NFS, with negligible calculation costs and immediate results, were developed as non-invasive surrogate fibrosis markers based on routine laboratory data and anthropometric and anamnestic parameters.[22-24] Both scores were validated for identifying patients whose NAFLD had a high probability of evolving into bridging fibrosis (F3) or cirrhosis (F4). FIB-4 scores >2,67 or NFS scores >0,676 identify people at high risk for advanced liver fibrosis (F3/F4) and are recommended for early evaluation of potential evolution towards severe fibrosis in people with T2DM, obesity, or metabolic syndrome. Several studies have shown an association between non-invasive-marker-based liver fibrosis and surrogate markers of arteriosclerosis, including carotid intima-media thickness and coronary calcium score.[43,44]

Indeed, FIB-4's and NPS's ability to predict increased cardiovascular risk was also tested in subjects without a well-defined NAFLD diagnosis. Long-term NAFLD effects on mortality were evaluated as part of the National Health and Nutrition Examination Survey led in the USA between 1988 and 1994.[45] After a mean 14.5-year follow-up, the US-based NAFLD diagnosis did not associate with increased mortality. Conversely, as non-invasively assessed through FIB-4, NFS, and APRI (AST/platelet count), liver fibrosis was predictive for cardiovascular mortality independently of other possible causes. More recently, in a 7.5-year follow-up study conducted in China in patients with stable coronary disease, the highest NFS and FIB-4 values were associated with a higher overall and cardiovascular mortality risk.[46] Starting from these results, the Authors proposed non-invasive hepatic fibrosis evaluation as a valuable and user-friendly tool to identify a long-term unfavorable prognostic factor in patients with coronary disease. Moreover, a posthoc analysis of an observational multicenter study on non-valvular atrial fibrillation patients from a Japanese registry showed an independent association of FIB-4-assessed liver fibrosis with cardiovascular and overall mortality, especially in patients at higher risk for adverse events.[46]

Limitations

This study analyzes surrogate markers of steato-fibrosis, which suggests extending the present observations to a similar population sample undergoing liver biopsy as the gold standard for comparison. However, the invasiveness of such a diagnostic approach would be difficult to overcome due to patients' hostility, thus hindering sample representativeness. However, albeit based on small clinical samples, enough evidence has accumulated in support of the strong relationship of those markers with liver biopsy results and of the reliability of an indirect, inexpensive, and clinically helpful approach through FLI, FIB-4, and NSF scores in large population samples.

Conclusion

Based on the abovementioned observations, dulaglutide treatment effects observed in our study are particularly interesting considering the significantly increased percentage of participants achieving a more favorable FIB-4 score (i.e., F0-F1-F2 cumulatively increased from 14.28 to 45.30% on the TG [p<0.001] while keeping virtually unchanged in the CG [passing from 15.48% to 15,42%, respectively). Analogously, the percentage of patients with an NFS score indicative of a low advanced fibrosis risk (i.e., <0.12) increased from 5.30% to 41.8% (p<0.001) in the TG while keeping unchanged in the CG (from 5.5% to 5.1%, p ns). Moreover, the concordance between FIB-4 and NSF scores was > 95%. Indeed, in add-on to empagliflozin, dulaglutide attenuated the inflammatory pathways and microbiome dysbiosis in non-diabetic mouse NASH models[47] and significantly reduced fatty liver content and g-GT circulating levels in a randomized controlled trial (D-LIFT trial).[48] The explanation for such an effect needs to be clarified and outside the scope of the present study, which, however, confirms results obtained with other GLP-1RAs and extends previous data on dulaglutide. In conclusion, dulaglutide treatment can decrease fat content and state-fibrosis markers in people with T2DM, steatosis, and altered cytolysis (AST/ALT) and cholestasis (gGT and ALP) markers, halt NASH progression towards more severe forms of hepatic damage and reduce the risk for cardiovascular events. Further studies are needed to understand the pathophysiologic mechanisms involved in such effects.

Acknowledgements

We are indebted to Dr. Paola Murano, General Manager of the Nefrocenter Research Network, for the effective and continuous support offered as complimentary, for the realization of the manuscript.

Funding

No funding or sponsorship was received for this study or the publication of this article. No payment was requested for publication and online posting costs. None of the authors or coworkers received funding or another type of payment for this paper. No funding was required for the "Editorial and Other Assistance".

Authorship

All named authors (Sandro Gentile, Felice Strollo, Giuseppina Guarino, Ersilia Satta, Emilia Martedì, Domenica Oliva, Carmine Martino, Clementina Brancario, Clelia Lamberti, Marco Corigliano, Gerado Corigliano, Marco Piscopo, Rosa Simonetti, Sara Colarusso, Miryam Ciotola, Pasqualino Calatola, Maria Rosaria Improta, e Alessandra Fusco) meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article taking responsibility for the integrity of the work as a whole and gave their approval for this version to be published.

Authorship contributions

Sandro Gentile and Felice Strollo designed the study and wrote the article. Giuseppina Guarino, Ersilia Satta, Emilia Martedì, Domenica Oliva, Carmine Martino, Clementina Brancasio, Clelia Lamberti, Marco Corigliano, Gerado Corigliano, Marco Piscopo, Rosa Simonetti, Sara Colarusso, Miryam Ciotola, Pasqualino Calatola, Maria Rosaria Improta, e Alessandra Fusco critically read and approved the paper. All authors contributed to data acquisition, critically assessed the results, and approved the final text. All collaborators critically read and approved the final text.

Compliance with ethics guidelines & institutional review board statement

Ours was a spontaneous, unconditioned study. The EC of the Vanvitelli University of Naples, Italy, approved the present study (protocol n. 1226/2019 of 07/06/2019). All participants signed informed consent, and data were processed anonymously according to good clinical practice guidelines. This study was conducted

in conformance with good clinical practice standards. The study was led in accordance with the Declaration of Helsinki 1975, as subsequent amendments. All followed procedures were in accordance with the ethical standards of the responsible committee on human experimentation (both institutional and national), and in accordance with usual clinical practice.

Informed consent statement

Written informed consent was obtained from all participants before enrollment.

Data availability statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosures

Sandro Gentile, Felice Strollo, Giuseppina Guarino, Ersilia Satta, Emilia Martedì, Domenica Oliva, Carmine Martino, Clementina Brancasio, Clelia Lamberti, Marco Corigliano, Gerado Corigliano, Marco Piscopo, Rosa Simonetti, Sara Colarusso, Miryam Ciotola, Pasqualino Calatola, Maria Rosaria Improta, e Alsessandra Fusco have no financial interests to declare concerning the present study.

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