Study Protocol

Study title: The effect of GLP-1 analogues on liver steatosis and fibrosis in diabetic and obese patients in a clinical setting
Study Acronym: GLP1_NAFLD
Protocol Number: 23141_GLP1_NAFLD
Protocol Version and Date: V 1.0 – 28/04/2023
ClinicTrials.gov Registry Number:
Investigational product: GLP-1 analogues
Sponsor: UZ Brussel
Coordinating/Principal Investigator: Hendrik Reynaert

PROTOCOL SIGNATURE PAGE

Protocol Version and date: V 1.0 – 28/04/2023
Protocol Title: The effect of GLP-1 analogues on NAFLD in diabetic and/or obese patients in a clinical setting
Sponsor: UZ Brussel / Vrije universiteit Brussel
Principal Investigator: Hendrik Reynaert

I agree:

- to assume responsibility for the proper conduct of this study
- to conduct the study in compliance with this protocol and any future amendments
- not to implement any deviations from or changes to the protocol without prior review and written approval from the Ethics Committee, except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements)
- that I am thoroughly familiar with the appropriate use of the investigational drug, as described in this protocol
- to ensure that all persons assisting me with the study are adequately informed about the investigational drug and their study-related duties and functions as described in the protocol
- that I am aware of and will comply with the current good clinical practice (GCP) guidelines and ethical principles outlined in the Declaration of Helsinki
- to conduct the study in accordance with all applicable laws and regulations

Printed name	Signature	Date
Hendrik Reynaert		Jette, 5/5/2023

1. Table of Contents

2. Synopsis

Information	
ClinicalTrials.gov:	pending
Official Title:	The effect of GLP-1 analogues on liver steatosis and fibrosis in diabetic and obese patients in a clinical setting
Objectives:	This study aims to measure the effect of GLP-1 analogues on NAFLD in a real-life clinical setting.
Investigational Product:	GLP-1 analogues
Study population:	Obese and/or diabetic patients with objectified NAFLD starting a GLP-1 analogue
Number of patients:	+- 100
Overview of study design:	Retrospective and prospective non-interventional study
Sponsor:	UZ Brussel
Country of recruitment:	Belgium
Inclusion Criteria:	• The patient is older than 18 years old. • The patient has obesity, defined as a BMI of 30 or higher; and/or the patient suffers from T2DM, defined as a twice measured sfG of > 125 mg/dl or higher, or a sfG of 100 - 125 mg/dl and a twice measured oral glucose tolerance test (OGTT) with a serum glucose (sG) of > 200 mg/dl, or higher after 2 hours, or a random sG of 200 mg/dl or higher in symptomatic patients. • The patient suffers from NAFLD in any stage, except cirrhosis. This has to be objectively diagnosed by either a CAP fibroscan (cutoff: > 238 dB/m); or by the calculated *Fatty Liver Index* (cutoff: > 60), at least one of which has to be positive. • The patient is starting a GLP-1 analogue as treatment for T2DM or obesity (standard of care); or the patient has started a GLP-1 analogue as treatment for T2DM or obesity (standard of care) 12 months prior to inclusion. • If the patient is part of the retro-prospective branch data collected 12 months prior include at least a (CAP) fibroscan, a blood sample (measuring AST, ALT, GGT, sfG, sfI, HbA1c, HDL, LDL, total cholesterol and platelets) and a physical examination (measuring blood pressure, waist circumference, weight and length).
Exclusion Criteria:	• The patient suffers from alcohol induced fatty liver disease. Macrocytic anemia; decreased vitamin B12 and folic acid; increased GGT, bilirubin, ferritin, TG and AST/ALT ratio can be used as serum markers of alcohol abuse. Interpretation of these results will be left to the patient's clinician and their clinical expertise. Alternatively a weekly alcohol consumption of 21 units for men and 14 units for women can be used as a cutoff. • The patient suffers from drug induced liver steatosis. Drugs warranting exclusion include: glucocorticoids, amiodarone, tamoxifen, methotrexate, valproate, tetracycline and chemotherapeutic agents. • The patient suffers from any other chronic liver disease. This will be checked trough lab test results in the patients file. • The patient has liver cirrhosis, defined as a fibroscan score of 14 kPa or more; or an elastography measurement of 10,5 kPa or more; or a *FIB-4 index* of > 2,67. • The patient is pregnant at time of enrolment or at any time during the study. • The patient refuses to agree to the informed consent.
Date of first enrolment:	Prospective inclusion: September 2023; Retrospective inclusion: September 2022
Target sample size:	+- 100

3. Protocol Version History

Version No.	Approval Date Lead EC	Release Date
V 1.0		**28/04/2023**

4. Sponsor/Coordinating Investigator Information

Coordinating Investigator: **Hendrik Reynaert**
Sponsor: **UZ Brussel**
Principal Investigator: **Hendrik Reynaert**
Co-investigators: **Yannis Polspoel**
Additional co-researchers: **NA**
Statistician: **NA**
Laboratory: **NA**
Pharmacy: **NA**
Study Coordinator: **Virgini Van Buggenhout**
Study sites and co-investigators: **UZ Brussel**

5. List of Abbreviations

Non-alcoholic fatty liver disease: **NAFLD**	Insulin resistance: **IR**
Advanced glycosylation end products: **AGEs**	Liver steatosis: **LS**
Alanine amino transferase: **ALT**	Lower limit of normal: **LLN**
Aspartate amino transferase: **AST**	Metabolic syndrome: **MetS**
Body mass index: **BMI**	Non-alcoholic steatohepatitis: **NASH**
De novo lipogenesis: **DNL**	Oral glucose tolerance test: **OGTT**
Free fatty acids: **FFA**	Serum fasting glucose: **sfG**
Gamma glutamyl transferase: **GGT**	Serum fasting insulin: **sfI**
Glucagon like peptide 1: **GLP-1**	Serum glucose: **sG**
Glucose dependent insulinotropic polypeptide: **GIP**	Type 2 diabetes mellitus: **T2DM**
Homeostatic model assessment of insulin resistance: **HOMA-IR**	Upper limit of normal: **ULN**

6. Introduction

Non-alcoholic fatty liver disease (NAFLD) has relatively recently become the leading cause of chronic liver disease in the West. (1) In Europe the prevalence is 23% which is predicted to grow further. Together with obesity and type 2 diabetes mellitus (T2DM), NAFLD is one of the major public health problems of the 21 century and therefore warrants further research. All three diseases are tightly associated with each other. (2, 3) Excess calorie intake leads to an increased triglyceride storage in adipocytes. Eventually this will cause insulin resistance (IR) predominantly through systemic low-grade inflammation, lipotoxicity and a changed adipokine balance[1]. (4) IR and excess calorie intake lead to abnormal lipid storage in hepatocytes which further increases local and systemic IR. Partially because of this fat deposits will form in pancreatic insulin-secreting beta cells leading to dysfunction. Other mechanisms of beta cell damage include glucotoxicity, lipotoxicity, inflammation (such as in obesity) and islet amyloid depositions. Impaired insulin secretion and IR eventually cause T2DM. All these mechanisms form self-reenforcing and interacting cycles[2]. Evidently there are complex and interlinked pathways connecting obesity, T2DM and NAFLD which are not yet fully understood.

The pathophysiology of NAFLD is complex and multifactorial. Liver steatosis (LS) is the first step in this process. (5) Lipolysis in adipocytes, increased by IR and excess fat stores in obesity, causes free fatty acids (FFA) to be offered to hepatocytes. (3, 6) Excess caloric intake, especially fructose and other carbohydrates, will further generate FFA. Lastly de novo lipogenesis (DNL), again strengthened by IR, increases the FFA acid load in liver cells. Triglyceride (TG) are formed and accumulate in fat droplets which cause LS, which in turn further contributes to IR and the thus to the development of T2DM. Non-alcoholic steatohepatitis (NASH) is the next step in pathogenesis of NAFLD. Lipotoxicity[3], inflammasome activation and an altered microbiome with increased epithelial permeability cause mitochondrial dysfunction, endoplasmic reticular stress and other hepatocytic injuries. (7) The resulting cellular dysfunction, inflammation, necrosis and apoptosis form NASH. Liver damage eventually starts fibrotic processes which can end in cirrhosis or hepatocellular carcinoma. Obesity and T2DM form aggravating factors for abovementioned disease processes trough an altered adipokine balance and formation of advanced glycosylation end products (AGEs) which activate fibrogenesis. Evidently LS, IR, T2DM and obesity have great influence on each other and have bidirectional effects.

GLP-1 analogues, such as semaglutide and liraglutide, are relatively novel drugs used for the treatment of T2DM and obesity. They work through different mechanisms to decrease glycemia and IR, and improve weight loss. (8, 9) Glycemic control is increased through the so-called incretin effect. In physiological conditions glucagon like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are post-prandially released from respectively intestinal L- and K cells. They then act on beta cells by stimulating the glucose-mediated release of insulin. Additionally GLP-1 inhibits the release of glucagon from pancreatic alpha cells, thus lowering glycemia. Weight loss is partially induced by delayed gastric emptying and decreased accommodation which increase satiation[4]. (10) Central actions include stimulation of satiety[5] and inhibition of food intake. IR mostly improves through weight loss, recent studies show however that GLP-1 can have direct effects on insulin sensitivity. (11)

[1] Increased resistine and TNF alpha, and decreased leptin and adiponectin lead to elevated IR.
[2] The exact pathophysiological processes and the order in which these take place are still partially unknown and have to be further explored.
[3] Lipotoxicity is mostly caused by oxidative stress mediated through FFA accumulation.
[4] Satiation refers to the feeling of fullness during and immediately after a meal acting as a mechanism to stop eating.
[5] Satiety refers to the feeling of fullness between meals acting as an inhibition to food intake.

Novel research shows that GLP-1 analogues can decrease LS and ameliorate liver histology. (12) Serum markers of glycemic control, IR, hepatic damage and inflammation all improved. (13) The exact mechanisms of action are not yet fully uncovered, but it is assumed that weight loss and improved IR play pivotal roles. Some direct actions on liver physiology have also been discovered. (9) Although these results seem promising the amount of studies focusing specifically on the effect of GLP-1 analogues on NAFLD, especially in a clinical setting, are limited. Factors such as lifestyle, age, polymedication, polypathology, compliance and social demographics have often been disregarded. Real patient populations, especially in hyper-diverse cities such as Brussels, are heterogeneous in this regard. The effects of GLP-1 analogues are likely different in this context. The real clinical impact has thus yet to be documented, which is why we propose this prospective non-interventional study evaluating the effects of GLP-1 analogues given to patients with NAFLD and suffering from obesity and/or T2DM. The goal is to verify if previously measured outcomes can be replicated in a real-life clinical setting with all the difficulties and confounding factors that accompany this. This study does not however aim to document all these factors, only to measure their end result.

7. Study Objectives

7.1 Primary Objective

This study aims to measure the effect of GLP-1 analogues on liver steatosis and fibrosis in diabetic and obese patients in a real-life clinical setting. Serum markers will be used to follow the evolution of liver damage and used to calculate the *Fatty Liver Index* and the *FIB-4 index* to evaluate steatosis and fibrosis respectively. Both pathogenic conditions will also be visualized and objectified using a (CAP) fibroscan.

7.2 Secondary Objectives

The effects of GLP-1 analogues on its primary indications, namely obesity and T2DM, will be investigated in a real-life clinical setting. Additionally the secondary effects on dyslipidemia will be evaluated. Parameters measured during a physical examination will be used to asses obesity. Serum markers will be used to follow the evolution of glycemic- and lipid control.

8. Methodology

8.1 Study Overview

This retrospective and prospective non-interventional study will be conducted in UZ Brussel. Participants will be recruited from consultations in both the diabetes- and obesity clinics. All patients with T2DM and/or obesity starting a GLP-1 analogue[6] are considered for inclusion. Data collection will either be retrospective or prospective.

In the first (retrospective) case physicians will screen their patient files (obtained through *Primuz*) for possible candidates using the inclusion and exclusion criteria (see 9.1 and 9.2). Most importantly the presence of NAFLD needs to be confirmed and GLP-1 analogues had to be started within 12 months prior to inclusion. If selected, the first consultation where GLP-1 analogues were started will act as starting point for data collection. If insufficient information exists to decide whether or not exclusion is necessary, additional measurement (see 8.2) will be performed during this first (and only) contact point. Data collected 12 months earlier will be used as baseline and additional data will be collected on the day of inclusion. All measurements are part of the standard of care.

In the second (prospective) case clinicians will evaluate possible inclusion (see 9.1 and 9.2) during the first consultation (where GLP-1 analogues are started). Data will be collected during this consultation and a follow-up 12 months later. All measurements are part of the standard of care.

8.2 Data Collection for Inclusion- and Exclusion Criteria

Most inclusion- and exclusion criteria (see 9.1 and 9.2) require information that cannot be procured solely based by anamnesis. On the one hand the presence of NAFLD needs to be confirmed using CAP fibroscan and/or the calculated *Fatty Liver Index*. Respective cutoffs are defined as > 238 dB/m and > 60. (14-16) On the other hand a fibroscan or elastography and blood tests are needed to obtain additional data regarding possible cirrhosis and liver diseases other than NAFLD, both of which are exclusion criteria**.** Lab test results in the file of the patient will be checked for chronic viral hepatitis B and C (HBsAg, anti-HBs Ab, anti-HCV Ab and HCV RNA), hemochromatosis (ferritin and transferrin saturation), alcoholic liver disease (Hb, Hct, MCV, B12, folic acid, GGT, AST/ALT ratio, bilirubin, ferritin and TG), auto-immune hepatitis and primary biliary cholangitis (IgG, IgM, ANA, ANCA, anti-mitochondrial antibodies and anti-smooth muscle cell antibodies). After signing the informed consent (see appendix) data collection can proceed. However if incoming results demonstrate exclusion, participation will be scraped and already collected data will be deleted. Abovementioned measurements are standard of care in UZ Brussel.

8.3 Data Collection

Data will be obtained from 2 consultations over a period of 12 months. In the case of the retrospective branch this includes data from 12 months prior to- and the day of inclusion. In the case of the prospective branch the first contact acts as starting point during which inclusion will be evaluated and data collection will start. The second contact (after 12 months) will solely be used for data collection

[6]All GLP-1 analogues available in Belgium are eligible. These include semaglutide, liraglutide, lixisenatide and dulaglutide.

(and the normal follow-up of the patient). Every measurement performed during these consultations is part of the standard of care in UZ Brussel.

Each consultation a physical examination will be performed to measure blood pressure, waist circumference, weight and length. BMI will be calculated using these last two. Weight, BMI and waist circumference will be used as markers of obesity.

Blood will be drawn to assess the evolution of serum markers of glycemic control (sfG, C-peptide and HbA1c), lipid control (LDL, HDL and total cholesterol) and liver damage (AST, ALT and GGT). (17-19) Platelets will also be measured. IR will be estimated using HOMA-IR. This model uses sfG and sfI.

Fibrosis and steatosis will be objectified using calculators (respectively the *FIB-4 index* and the *Fatty Liver Index*) and a (CAP) fibroscan or elastography. Both absolute and categorial[7] values will be recorded. The *FIB-4 index* uses AST, ALT, platelet count and age. (20) In patients < 49 years old a *FIB-4 index* of < 1,05 is categorized as low-, 1,05 – 1,21 as intermediate- and > 1,21 as high risk for fibrosis. (21) In patients 50 - 59 years old a *FIB-4 index* of < 1,24 is categorized as low-, 1,25 – 1,96 as intermediate- and > 1,96 as high risk for fibrosis. In patients 60 – 69 years old a *FIB-4 index* of < 1,88 is categorized as low-, 1,88 – 2,67 as intermediate- and > 2,67 as high risk for fibrosis. In patients > 70 years old a *FIB-4 index* of < 1,95 is categorized as low-, 1,95 – 2,67 as intermediate- and > 2,67 as high risk for fibrosis. The *Fatty Liver Index* uses GGT, TG, BMI and waist circumference to calculate the risk of NAFLD. (16) A score < 30 rules out- and a score > 60 rules in NAFLD. This study defines fibroscan measurements of 2 - 7 kPa as F0 - F1 (no to mild fibrosis), 7 - 10 kPa as F2 (moderate fibrosis), 10 - 14 as F3 (severe fibrosis), and > 14 kPa as F4 (cirrhosis). This study defines elastography measurements of < 7kPa (< 1,53 m/s) as F0-F1, 7 - 9,5 kPa (1,53 – 1,78 m/s) as F2, 9,5 – 10,5 (1,78 – 1,87 m/s) as F3 and > 10,5 (> 1,87 m/s) as F4. This study defines CAP fibroscan measurements of < 238 dB/m as S0 (no steatosis), 238 - 260 dB/m as S1 (mild steatosis), 260 - 290 dB/m as S2 (moderate steatosis) and > 290 dB/m as S3 (severe steatosis). Abovementioned cutoffs were chosen because they are the standards in the institution (UZ Brussel) in which this study will take place.

8.4 Data Protection and Management

All data will be pseudonymized. After inclusion physicians will add their patients to a protected worksheet in *Primuz* which will only be accessible to the primary investigators. Each participants will receive a unique subject number. Data will be retrieved from *Primuz* and recorded on a standardized excel sheet (see appendix) which will only differentiate between patients using subject numbers. No information will be recorded that could identify a participant's identity.

[7] No categorization exist for the *NAFLD liver fat score*.

8.5 Data Processing

Once all data is collected it will be exported to SPSS to be analyzed. Each variable will be compared to itself at previous time points using the student's T-test with alpha = 0,05. Changes in categorial values will be evaluated using the Pearson's $\chi 2$ test with alpha = 0,05. Evidently normal distribution of data will first be verified.

9. Selection of Subjects

9.1 Selection of Study Population

Participants will be recruited from consultations in the diabetes- and obesity clinic in UZ Brussel. All diabetic and/or obese patients with objectified NAFLD starting a GLP-1 analogue, or whom have started a GLP-1 analogue 12 months prior, will be considered as possible candidates. Inclusion is final after it is established that patients comply to all inclusion- and exclusion criteria and they agree to the informed consent. Patients can withdraw at any point during the study.

9.2 Inclusion Criteria

- The patient is older than 18 years old.
- The patient has obesity, defined as a BMI of 30 or higher;
  and/or the patient suffers from T2DM, defined as a twice measured sfG of > 125 mg/dl or higher, or a sfG of 100 - 125 mg/dl and a twice measured oral glucose tolerance test (OGTT) with a serum glucose (sG) of > 200 mg/dl, or higher after 2 hours, or a random sG of 200 mg/dl or higher in symptomatic patients.
- The patient suffers from NAFLD in any stage, except cirrhosis, objectively diagnosed by either a CAP fibroscan (cutoff: > 238 dB/m); or by the calculated *Fatty Liver Index* (cutoff: > 60), at least one of which has to be positive.
- The patient is starting a GLP-1 analogue as treatment for T2DM or (standard of care);
  or the patient has started a GLP-1 analogue as treatment for T2DM or obesity (standard of care) 12 months prior to inclusion.
- If the patient is part of the retro-prospective branch data collected 12 months prior include at least a (CAP) fibroscan, a blood sample (measuring AST, ALT, GGT, sfG, sfI, HbA1c, HDL, LDL, total cholesterol and platelets) and a physical examination (measuring blood pressure, waist circumference, weight and length).

9.3 Exclusion Criteria

- The patient suffers from alcohol induced fatty liver disease. Macrocytic anemia; decreased vitamin B12 and folic acid; increased GGT, bilirubin, ferritin, TG and AST/ALT ratio can be used as serum markers of alcohol abuse. Interpretation of these results will be left to the patient's clinician and their clinical expertise. Alternatively a weekly alcohol consumption of 21 units for men and 14 units for women can be used as a cutoff. (22)
- The patient suffers from drug induced liver steatosis. Drugs warranting exclusion include glucocorticoids, amiodarone, tamoxifen, methotrexate, valproate, tetracycline and chemotherapeutic agents. (23)
- The patient suffers from any other chronic liver disease. This will be checked trough lab test results in the patients file.
- The patient has liver cirrhosis, defined as a fibroscan score of 14 kPa or more;
  or an elastography measurement of 10,5 kPa or more;
  or a *FIB-4 index* of > 2,67.
- The patient is pregnant at time of enrolment or at any time during the study.
- The patient refuses to agree to the informed consent.

10. Ethical Considerations

10.1 Ethical conduct of the study

10.1.1 Declaration of Helsinki

This study will be performed in accordance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and local laws and regulation as recommended by the European Community.

10.1.2 Ethics Committee

Approval will be obtained from the appropriate regulatory authority in Belgium prior to the start of the study, in this case the Ethical Committee of UZ Brussel. Investigators and personnel are responsible for acting in accordance with their local regulatory requirements. The study will start on-site only after approval by the local ethical committee.

10.2 Informed Consent

All eligible patients should provide a signed and dated informed consent (see appendix) before inclusion into this study.

10.3 Subject and Study Data Protection

All data will be pseudonymized. After inclusion physicians will add their patients to a protected worksheet in *Primuz* which will only be accessible to the primary investigators. Each participants will receive a unique subject number. Data will be retrieved from *Primuz* and recorded on a standardized excel sheet (see appendix) which will only differentiate between patients using subject numbers. No information will be recorded that could identify a participant's identity.

10.4 Conflict of Interest

This study reports no conflict of interest. All participating investigators will list their possible conflicts of interests.

11. Finance and Insurance

This academic study is sponsored by UZ Brussel. All data collection is part of the standard of care. No additional costs will be charged. No compensation will be provided for participation.

Adequate insurance coverage for medical professional and/or malpractice liability and general liability will be maintained in full force and effect during the term of this study, and following termination of the study to cover any claims arising from the trial. Additionally in accordance to art. 29 of Belgian Law relating to experiments on human persons, dated May 7th, 2004 an insurance contract will be obtained in order to cover Trial Participants.

12. Reporting and Dissemination

Reporting and dissemination requires prior agreement of the principle investigator.

13. Conflict of interest statement

This study reports no conflicts of interest. All participating investigators will list their possible conflicts of interests.

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