CLINICAL TRIAL PROTOCOL

A neuropsychobiological approach to optimize patient selection for GLP-1-based pharmacotherapy for weight management across the binge eating spectrum

SEM-BEAT

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Sponsor

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This Clinical Trial Protocol also refers to the Master Platform Protocol (EUDAMED_ CIV-25-06-053398, Coordinating PI: [redacted]) "*Master protocol for all Positron Emission Tomography studies using the NeuroExplorer PET/CT at UZ/KU Leuven*" for those study parts where the investigational device the "NeuroEXPLORER PET/CT" is used. For details see Appendix 2.

Confidentiality Statement

The information in this document is strictly confidential and is available for review to Investigators, potential Investigators and appropriate Ethics Committees, Institutional Review Boards or Competent Authorities. No disclosure should take place without written authorization from the Sponsor.

LIST OF PARTICIPATING SITES

List Of Participating Sites	Principal Investigator
UZ Leuven, Herestraat 49, 3000 Leuven (B)	Prof. Dr. Roman Vangoitsenhoven

SIGNATURES

Title: A neuropsychobiological approach to optimize patient selection for GLP-1-based pharmacotherapy for weight management across the binge eating spectrum

Protocol: SEM-BEAT

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, and agree to conduct the Trial in compliance with the approved protocol, and will adhere to: the principles outlined in the requirements for the conduct of clinical Trials in the EU as provided for in the EU Clinical Trial Regulation 536/2014 (CTR) and any subsequent amendments thereto, the ICH guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2017 related to clinical Trials on medicinal products for human use, the EU General Data Protection Regulation 2016/679 (GDPR), the Belgian law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data, the Belgian Law of August 22nd 2002 on patient rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Trial, without prior written consent of the Sponsor.

The undersigned also commit to making the findings of the Trial publicly available through publication and/or other dissemination tools, in accordance with this protocol and applicable regulations, without any unnecessary delay and to provide an honest, accurate and transparent account of the Trial; and to explain any discrepancies or deviations from the approved Trial protocol.

Coordinating Investigator

Prof. Dr. Roman Vangoitsenhoven	……	……
Name & Title	Signature	Date

Principal Investigator (Participating Site) *(in case of monocentric Trial, the Principal Investigator is the same as the Coordinating Investigator)*

Prof. Dr. Roman Vangoitsenhoven	……	……
Name & Title	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
(e)CRF	(electronic) Case Report Form
ADHD	Attention deficit hyperactivity disorder
AE	Adverse Event
AESI	Adverse Event of Special Interest
Aib	Aminoisobutyric acid
ANS	Autonomic nervous system
AR	Adverse Reaction
ASL	Arterial spin labelling
BBB	Blood-brain barrier
BE	Binge eating
BED	Binge eating disorder
BES	Binge Eating Scale
BIS/BAS	Behavioral Inhibition System/Behavioral Activation System
BSCFB	Blood-cerebrospinal fluid barrier
CA	Competent Authority
CBT	Cognitive behavioral therapy
CI	Coordinating Investigator
CM	Concomitant Medication
CSR	Clinical Study Report
CTIS	Clinical Trial Information System
CTP	Clinical Trial Protocol
CTR	EU Clinical Trial Regulation 536/2014
D2R	D_2 receptor
DA	Dopamine
DEBQ	Dutch Eating Behavior Questionnaire
DMP	Data Management Plan
DPA	Data Processing Annex
DPP-4	Dipeptidyl peptidase-4
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
EC	Ethics Committee
ECG	Electrocardiogram
ED	Eating disorder
EDE-Q	Eating Disorder Examination Questionnaire
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked immunosorbent assay
EoT	End of Trial
ESM	Experience Sampling Methodology

EU	European Union
FANC	Federal Agency for Nuclear Control
FFQ	Food Frequency Questionnaire
FID	Food Incentive Delay task
FPFV	First Patient First Visit
GAD-7	Generalized Anxiety Disorder-7
GC-MS	Gas chromatography-mass spectrometry
GCP	Good Clinical Practice (latest version of ICH E6)
GCP	Good Clinical Practice
GDPR	EU General Data Protection Regulation 2016/679
G-FCQ-S	General Food Craving Questionnaire (state)
G-FCQ-T	General Food Craving Questionnaire (trait)
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1 RA	Glucagon-like peptide-1 receptor agonist
HbA1c	Hemoglobin A1c
HR	Heart rate
HRV	Heart rate variability
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council on Harmonisation
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ITI	InterTrial intervals
JCI	Joint Commission International
LaBGAS	Laboratory for Brain-Gut Axis Studies
LASSO	Least Absolute Shrinkage and Selection Operator
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LFPQ	Leeds Food Preference Questionnaire
LPLV	Last Patient Last Visit
LSRRM	Linearized simplified reference region model
MAH	Marketing Authorisation Holder
MAOIs	Monoamine oxidase inhibitors
MEN2	Multiple endocrine neoplasia type 2
MI	Myo-inositol
MID	Monetary Incentive Delay Task
MINI-S	Mini International Neuropsychiatric Interview Simplified
MMTT	Mixed Meal Tolerance Test
MP	Monitoring Plan
MRI	Magnetic Resonance Imaging
NAA	N-acetyl-L-aspartate

PANAS	Positive and Negative Affect Schedule
PCR	Principal components regression
PET	Positron Emission Tomography
PFS	Power of Food Scale
PHQ-9	Patient Health Questionnaire-9
PI	Principal Investigator (Participating Site)
PRO	Patient Reported Outcome
PYY	Peptide YY
RedCap	Research Electronic Data Capture
RIA	Radioimmunoassays
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SCFA	Short-chain fatty acids
SEES	Salzburg Emotional Eating Scale
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SP	Snack points
SPM	Statistical Parametric Mapping
SSRIs	Serotonin reuptake inhibitors
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2DM	Type 2 diabetes mellitus
TMF	Trial Master File
UPC	University Psychiatric Centre
VAS	Visual Analogue Scales
vmPFC	Ventromedial prefrontal cortex
VTA	Ventral tegmental area
WHO	World Health Organization
WOCBP	Women Of Child Bearing Potential

FUNDING AND THIRD PARTIES

Funder or service provider	Type of Support
Internal fund (BOF): KU Leuven C2 grant (C2M/25/041K)	Financial
[redacted]	Blood serum sample analysis

Participants will receive financial compensation for participation in the study. Compensation will be provided per completed study visit day or assessment period, as follows:

- €75 per MRI-MMTT study visit day (approximately 6-7 hours in total), reflecting the duration of the visit, fasting requirements, repeated blood sampling and MRI procedures;
- €100 per PET scan day (approximately 4-5 hours), reflecting the duration of the visit, radiotracer administration, radiation exposure associated with PET imaging, repeated blood sampling and the discomfort associated with the requirement to lie still in the scanner for prolonged periods;
- €50 for the whole ESM and wearable daily-life assessment period, reflecting the burden associated with monitoring at home and repeated daily assessments.

If the participant withdraws from the study, the participant will receive compensation proportional to the completed study visits and assessments up to the time of withdrawal. Partial participation will not result in loss of compensation for completed study activities.

Participants who do not undergo PET imaging may receive a maximum total compensation of €200. Participants undergoing two PET scan visits may receive a maximum total compensation of €400.

If contraception is required for a participant in the context of this study, reimbursement of €40 per 3 months may be provided by the Sponsor. This reimbursement applies for the duration of the study until 2 months after the last dose of semaglutide.

ROLES AND RESPONSIBILITIES

The Principal Investigator (PI) is responsible for the conduct of the Trial at his/her Participating Site, and for protecting the rights, safety and well-being of the Trial participants. As such the PI must ensure adequate supervision of the Trial conduct at the Participating Site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified Trial-related duties. The PI will ensure that adequate training is provided and documented for all Trial staff, prior to conducting assigned Trial-related activities.

It is the Coordinating Investigator's (CI's) responsibility to supervise the general conduct (e.g. Trial progress, communication, protocol training and support of the participating sites, annual reporting to the Ethics Committee (EC), end of Trial notification(s) and results reporting, …) of the Trial. The CI fulfils both Investigator and Sponsor responsibilities, as outlined in latest version of the International Council on Harmonisation – Good Clinical Practice (ICH-GCP) E6 and applicable regulations.

PI and CI shall each be referred to as «Investigator(s)».

TRIAL SYNOPSIS

Title of clinical Trial («Trial»)	A neuropsychobiological approach to optimize patient selection for GLP-1-based pharmacotherapy for weight management across the binge eating spectrum
Protocol Short Title Acronym	SEM-BEAT
Trial Phase (I, II, III, IV)	Phase IV
Sponsor name	UZ Leuven
Coordinating Investigator	Prof. Dr. Roman Vangoitsenhoven
Contact Address CI	- KU Leuven, Clinical and Experimental Endocrinology, Herestraat 49, 3000, Leuven (B) - UZ Leuven, Endocrinology, Herestraat 49, 3000, Leuven (B)
Contact Email CI	
Contact Phone CI	
EU CT number	2026-526016-36
Other public database nbr	N/A
Medical condition or disease under investigation	Obesity
Trial rationale	This Trial is designed to provide a sound scientific foundation for optimizing patient selection for GLP-1-based pharmacotherapy as a treatment for obesity across the BE spectrum, as well as to identify its mechanisms of action.
Primary objective	To identify for whom semaglutide works best, i.e., to predict heterogeneity of treatment response based on baseline characteristics. Specifically, we aim to determine whether the baseline BES score predicts the change in BMI after semaglutide treatment and whether other baseline eating behavior characteristics (excluding the BES score) predict the change in BES score after semaglutide treatment.
Secondary objective(s)	To investigate how semaglutide works. To examine whether semaglutide's psychobiological mechanisms of action differ along the BE spectrum. To develop a multivariate prediction model for successful weight and BE severity reduction in patients with obesity across the BE spectrum.
Trial Design	Prospective, single-center, longitudinal, interventional Trial
Endpoints	Primary endpoints: change in BMI and BES score after semaglutide treatment (baseline vs at 6 months of semaglutide treatment). Secondary endpoints: change in waist/hip ratio, body composition, gut hormone blood levels, DA blood and urinary levels, appetite-related sensations, DA release in the brain in response to food reward, neural responses to food reward, food liking, food wanting, food craving, emotional state (stress, positive affect, negative affect and negative urgency), HR, HRV, hair cortisol levels, SCFA blood levels, cytokine blood levels, BBB/BSCFB integrity/permeability and neuroinflammation

	after semaglutide treatment (baseline vs at 6 months of semaglutide treatment).
Sample Size	*n=32*/group, 3 groups (low, intermediate and high BES score), *n=96* in total
IMP, dosage and route of administration	Semaglutide 3,2 mg/ml (Wegovy®) Starting dose is 0.25 mg once a week, escalated every four weeks until the maximal dose of 2.4 mg per week or the highest tolerated dose is reached, which will be maintained until the end of the Trial Route of administration: subcutaneous in the abdomen, thigh or upper arm
Active comparator product(s)	N/A
Maximum duration of treatment and Follow Up of a Participant	Duration of the treatment: 6 months
Date anticipated First Patient First Visit (FPFV)	1 July 2026
Date anticipated Last Patient Last Visit (LPLV)	31 December 2028

TRIAL FLOWCHART

Schedule of Events – Trial specific Procedures / Assessments

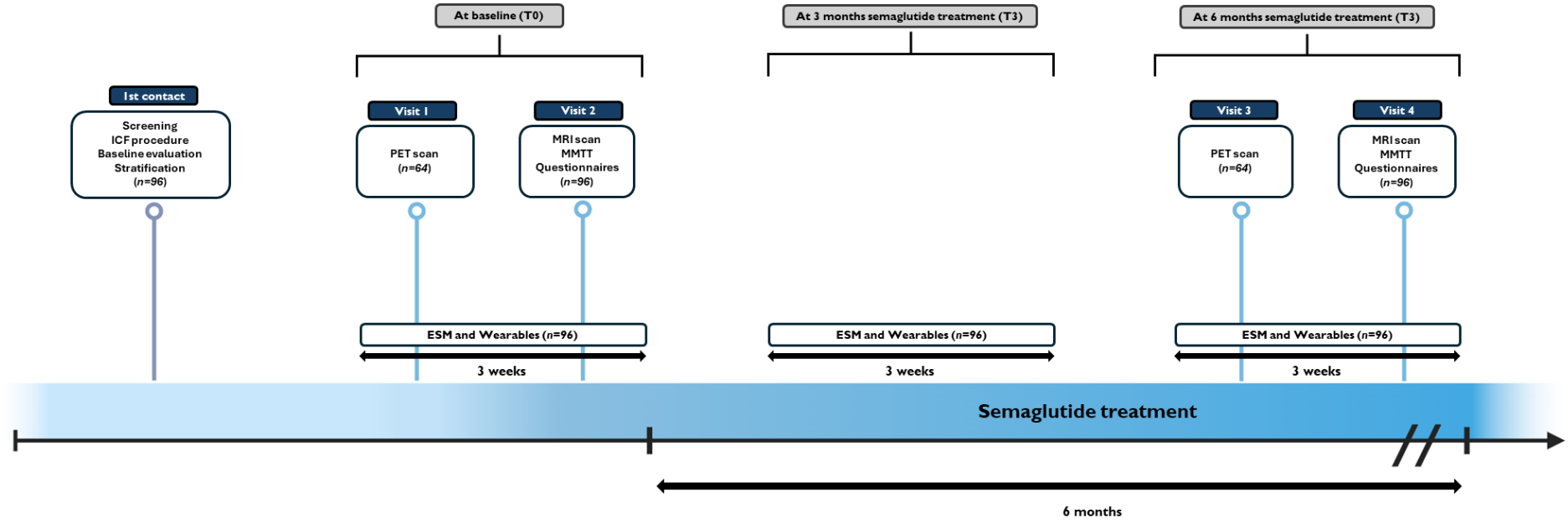
Procedures/ Assessment	**Screening, ICF procedure & stratification**	**Baseline assessments**		**During the treatment period (6 months)**		**Assessments at end of treatment**	
Visits / Contacts	First contact during a routine visit (*n*=96)	Visit 1: PET scan visit (*n*=64)	Visit 2: MRI-MMTT visit (*n*=96)	Telephone contact (*n*=96)	Remote pregnancy testing (only WOCBP)	Visit 3: PET scan visit (*n*=64)	Visit 4: MRI-MMTT visit (*n*=96)
Timing (months)	-1 months	-0.5 months	0 months	3 months	monthly (x1)	5.5 months	6 months
Visit Window (days)	±14 days	±14 days	±14 days	±7 days	±7 days	±14 days	±14 days
Informed consent [1]	X						
Inclusion / Exclusion criteria	X						
Stratification based on BES score	X						
Demographics	X						
Medical, Surgical history	X						
Medication use	X						
Weight / Height / BMI	X						
Waist-to-hip ratio	X						
Body composition	X						
Comorbidity status	X						
Laboratory tests							
Blood sample for analyses UZ Leuven [2]	X						
Urine drug test	X	X	X			X	X
24h urine analysis [3]			X				X
Blood sampling for analysis of metabolic hormones and peripheral dopamine concentrations		X	X			X	X
Blood sampling for analysis of SCFA and cytokine concentrations			X				X
Hair sample for analysis of chronic hair cortisol			X				X
Pregnancy test (urine)[4]	X	X			X	X	

Brain imaging							
MRI scan			X				X
Multi-echo ASL			X				X
MR spectroscopy			X				X
PET scan [5]		X				X	
Questionnaires and diaries							
The Patient Health Questionnaire-9 (PHQ-9)	X		X				X
The General Anxiety Disorder-7 (GAD-7)	X		X				X
The Binge Eating Scale (BES)	X		X				X
Mini International Neuropsychiatric Interview Simplified (MINI-S)	X						
Digital food diary [6]		X	X			X	X
The Eating Disorder Examination Questionnaire (EDEQ)			X				X
The Food Frequency Questionnaire (FFQ)			X				X
The Dutch Eating Behavior Questionnaire (DEBQ)			X				X
The Power of Food Scale (PFS)			X				X
The General Food Craving Questionnaire (Trait and State) (G-FCQ-T & G-FCQ-S)			X				X
The Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) questionnaire			X				X
The Leeds Food Preference Questionnaire (LFPQ)			X				X
The Positive and Negative Affect Schedule (PANAS)			X				X
The Salzburg Emotional Eating Scale (SEES)			X				X
The Self-Report Habit Index (SRHI)			X				X
Visual analogue scales (VAS)		X	X			X	X
Ambulatory assesments							
ESM questionnaires [7]		X	X	X		X	X
Continious measurements of HR and HRV via wearables [8]		X	X	X		X	X
Trial drug management							
Trial drug treatment (sc)			X[9]	X	X	X	X[10]
Trial drug dispensation (sc)			X[9]				
Trial drug accountability (sc)			X[9]	X	X	X	X[10]
Safety assesments							
Reason for discontinuation		X	X	X		X	X

(Serious) Adverse event (S)(AE) assessment		X	X	X		X	X
Concomitant Medication (CM)		X	X	X		X	X

1 : Informed Consent be obtained prior to performing any other Trial-related procedures.
2 : Only if a participant has not had a routine blood sample within 4 months prior to screening. If a participant has had a routine blood sample within 4 months prior to screening, the results of this previous routine blood panel will be used to evaluate the in- and exclusion criteria.
3 : On the day prior to a MRI-MMTT visit, participants will collect urine for 24h.
4 : At the screening visit, at PET scan visits and once per month throughout the Trial, WOCBP are required to take a urine pregnancy test to rule out the possibility of pregnancy, as the effects of semaglutide on a developing fetus are not known and to prevent any fetal exposure to radiotracers.
5: Only a subsample of 64 patients will undergo PET scanning
6 : On the three days before a MRI-MMTT visit, participants will be asked to fill in a digital food diary. A subsample of 64 participants will also have to do this on the two days before, the day of and the day after the PET visits.
7 : Three bursts of data collection (T0, T3, T6) each lasting 3 weeks, where data is only collected on Thursday, Friday and Saturday.
8 : Three bursts of data collection (T0, T3, T6) each lasting 3 weeks, where data is continuously collected via wearables.
9 : At the end of the baseline MRI–MMTT visit, all semaglutide administration procedures will be explained, the semaglutide pen injectors for the treatment period will be provided and the first semaglutide dose will be injected.
10 : This MRI–MMTT visit should be scheduled within 6 days after the participant's last semaglutide injection.

Standard of care
Specifically for the trial



1 Background, Rationale and Risk Assessment

Background and rationale

Obesity is a chronic, multifactorial disease characterized by excessive fat accumulation that progressively impairs health and functioning, contributing to numerous health complications and increasing mortality risk (1,2). According to the World Health Organization (WHO), approximately 16% of adults worldwide were living with obesity in 2022, more than twice the prevalence recorded in 1990 (3). This ongoing global rise is expected to continue accelerating in the coming decades, posing a growing threat to public health and imposing a substantial economic burden on societies worldwide (4).

The etiology of obesity extends far beyond a simple imbalance between energy intake and expenditure. From an evolutionary perspective, humans have a natural preference for sweet and fatty foods, and store body fat efficiently. While these adaptations were advantageous in ancestral periods of scarcity, in today's sedentary societies with constant access to energy-dense foods, they contribute to excessive fat accumulation and hinder weight loss (5–7). Besides genetic predisposition, obesity is driven by a variety of other person-specific factors, including (food-related) cognitions, emotions, psychosocial stress and social context, most of which act, at least partly, on eating behavior (6). Hence, individual differences in the psychobiological mechanisms that regulate eating behavior likely explain why some people are more prone to overeating and weight gain than others (8,9). Consequently, interventions that target only motivation or behavior often fail to achieve sustained weight reduction, as they do not address the underlying psychobiological mechanisms that maintain excess body weight (10,11). Effective obesity treatments should therefore focus on both (neuro)biological and psychological drivers of eating behavior at the individual level.

The pharmacological treatment of obesity has advanced markedly in recent years, most notably with the introduction of glucagon-like peptide-1 receptor agonists (GLP-1 RAs). Originally developed for the management of type 2 diabetes mellitus (T2DM), GLP-1 RAs were found to induce dose-dependent weight loss, rapidly becoming a primary therapeutic indication, leading to global shortages (12,13). Unlike previous pharmacotherapies, GLP-1 RAs represent the first safe, non-invasive therapy capable of supporting sustained weight loss while mitigating the "jo-jo effect" commonly observed with caloric restriction and lifestyle modifications (14). The weight-lowering effects of GLP-1 RAs are primarily attributed to enhanced homeostatic satiety signaling via GLP-1 receptor activation, acting through both direct and indirect central mechanisms (15). In humans, GLP-1 RAs reduce total daily ad libitum energy intake, particularly by decreasing consumption of high-fat and sweet foods (16). This selective reduction of palatable food intake suggests that GLP-1 RA actions extend beyond homeostatic circuitry. Indeed, preclinical studies indicate that GLP-1R activation within reward-related neural circuits selectively suppresses intake of energy-dense foods without affecting consumption of standard diets (17). Animal studies suggest that GLP-1 RAs modulate dopaminergic signaling within the mesolimbic reward pathway (18). For example, Kooij et al. (2024) found that the GLP-1 RA semaglutide increased dopamine (DA) activity in the ventral tegmental area (VTA) during consummatory food reward, but not during the anticipatory phases (19). However, such mechanistic insights in humans remain largely unexplored and are yet to be established.

Despite the proven efficacy, clinical evidence shows considerable variability in individual response to GLP-1 RA treatment, as total weight loss was similar regardless of differences in the maximally tolerated maintenance dose (20). While approximately 30% of patients achieve >20% weight loss, 15-30% experience insufficient weight loss with semaglutide, despite optimal dosing (21). Understanding the determinants of this variability is critical for developing personalized treatment strategies (22). Emerging evidence points to eating behavior as a key predictor of treatment responsiveness. For example, individuals with T2DM exhibiting higher emotional or external eating were less sensitive to GLP-1RA effects, whereas those with restrained eating may increase sensitivity to the effects of GLP-1 RAs (23).

Among maladaptive eating behaviors, binge eating (BE) appears particularly relevant. BE, characterized by recurrent episodes of consuming an unambiguously large amount of food with a sense of loss of control, is the hallmark symptom of binge eating disorder (BED), which is the most common eating disorder (ED) worldwide (24). Affecting up to half of individuals with obesity, BE strongly predicts caloric intake across the BMI spectrum and may thus play a critical role in shaping responses to GLP-1 RAs (25–27). Despite its high prevalence, BE is underdiagnosed in obesity, due to underrecognition by healthcare professionals and guilt or shame preventing patients from disclosing symptoms (28). Conversely, patients with BED are three to six times more likely to develop obesity, suggesting shared psychobiological drivers between the two conditions (29). Despite this, individuals with BE(D) are typically excluded from clinical weight-loss Trials, including those evaluating GLP-1 RAs. Cognitive behavioral therapy (CBT) is the main treatment for BE(D), improving BE behavior and emotional state in over 50% of patients (30). However, CBT does not target weight loss and its long-term effects on both are disappointing (30). Hence, more effective therapies for BE(D) in the context of obesity need to be explored, but a better understanding of its psychobiological underpinnings is required to develop them.

Given the interindividual variability in GLP-1 RA response, the potential role of eating behaviors, particularly BE, in driving this variability, the exclusion of individuals with EDs (like BED) from clinical Trials and lack of systematic evaluation of eating behaviors (including BE), we believe there is a true therapeutic blind spot. Despite these observations, the motivational and psychobiological mechanisms through which GLP-1RAs influence eating behavior, including BE, remain poorly understood, limiting our ability to predict individual treatment response. Therefore, this project aims to provide a scientific basis to transition from a one-size-fits-all approach to more personalized treatment plans, factoring in BE behavior and its psychobiological mechanisms. Specifically, the study will examine the mechanisms of action through which GLP-1 RAs influence the motivational and psychobiological processes underlying eating behavior and weight regulation, and whether these effects vary according to BE severity. Such insights are expected to improve patient selection, optimize the use of GLP-1 RAs amid current supply constraints and ultimately enhance clinical outcomes for both individuals with obesity and BE.

Investigating GLP-1 RAs effect on psychobiological processes underlying eating behaviour and weight regulation in binge eating in the context of obesity

This study will investigate GLP-1 RA effects on three key psychobiological processes shaping eating behavior and weight regulation.

First, homeostatic hunger and satiety signaling regulates food intake according to metabolic needs via gut-brain hormones (31). In obesity, this signaling is often disrupted, leading to reduced satiety (32).

Second, eating behavior is strongly influenced by reward-related processes in the brain's hedonic circuitry (33). Central to this process is the mesocorticolimbic dopaminergic signaling, which encodes the rewarding properties of food and reinforces food-seeking behavior (34). In obesity and BE, individuals show altered food reward sensitivity and weakened inhibitory control, presumably due to altered food-induced DA signaling, though research on this remains limited and inconsistent (35–39).

Third, negative emotional states, such as stress and negative affect, promote aberrant eating behaviors, such as BE (8,40). Moreover, negative emotional states and emotion regulation difficulties are a transdiagnostic risk and perpetuating factor for eating and weight disorders (41,42). Elevated levels of chronic cortisol, an important stress hormone, are associated with obesity (43,44). How chronic cortisol levels relate to food intake in obesity is unclear, but acute cortisol reactivity to stress predicts stress-induced food intake in obesity, but not in healthy weight controls (45).

Relatively little research has examined how emotional states interact with neurobiological reward system mechanisms. However, recent evidence showed that stress-induced DA release in the ventromedial prefrontal cortex (vmPFC) impacts reward system function (fronto-striatal functional connectivity), which in turn relates to negative urgency (i.e. a tendency to act rashly while distressed)(46). This suggests that high negative affect and stress may enhance the rewarding value of food by interfering with the hedonic

DA circuitry (37). Two pathways are thought to be involved in mediating this relationship: an inflammatory pathway, as chronic psychosocial stress can drive low-grade systemic inflammation and neuroinflammation, which, in turn, impacts DA signaling; and an endocrine pathway given that acute stress has been shown to increase ghrelin and leptin, metabolic hunger/satiety hormones that also impact the DA reward pathway (47–51). Furthermore, gut microbiota and their microbial metabolites, short-chain fatty acids (SCFAs), are thought to modulate both pathways (52).

Together, these homeostatic, hedonic and emotional mechanisms are presumed to interact and drive eating behavior. Thus, these mechanisms may serve as predictors of individual response to GLP-1 RA treatment.

Risk/benefit assessment

To investigate GLP-1 RA signaling, this study focuses on semaglutide, a potent agent in its class and is generally well-tolerated, with transient, mild gastrointestinal (GI) side-effects, including nausea, vomiting and diarrhea (53,54). All potential side-effects will be closely monitored throughout the study to ensure timely detection and management.

Risks regarding treatment with semaglutide

GI side-effects such as nausea, vomiting, diarrhoea, abdominal pain and constipation have been among the most frequently reported side-effects with semaglutide (55). These symptoms are generally mild to moderate in severity and often transient, although in some participants they may be severe and may lead to dehydration. In rare cases, dehydration may contribute to deterioration of renal function, especially in participants with impaired renal function. Clinical trial data with semaglutide 2.4 mg demonstrate that GI adverse events are most frequent during dose escalation and are usually transient in nature (56). The risk is minimized by starting at a low dose followed by gradual dose-escalation (cf. Section 5).

Because semaglutide delays gastric emptying, participants with severe gastroparesis or severe GI motility disorders will be excluded. Delayed gastric emptying may rarely lead to more severe GI complications, including severe constipation or intestinal obstruction. Participants will be advised to maintain adequate fluid intake, eat more slowly and eat smaller meals. GI symptoms will be actively assessed at each study visit, including nausea, vomiting, abdominal pain and bloating. In case of intolerable GI SE dose escalation may be delayed, symptomatic treatment (e.g., antiemetic or antidiarrheal medication), temporary treatment interruption or discontinuation may be considered by the investigator.

Delayed gastric emptying may increase the risk of residual gastric contents in patients undergoing general anaesthesia or deep sedation. Cases of pulmonary aspiration have been reported in patients receiving GLP-1 receptor agonists undergoing general anaesthesia or deep sedation (55). Therefore, participants who have major surgery scheduled during the duration of the study will be excluded from the Trial. Participants will be informed about this risk and will be instructed to inform their healthcare providers that they are receiving semaglutide prior to any emergency or unplanned procedures requiring general anaesthesia or deep sedation. Peri-procedural management will be determined by the treating anaesthetist according to clinical judgement and applicable guidance.

Delayed gastric emptying could potentially influence the absorption of concomitantly administered oral medicinal products, particularly medicinal products requiring rapid GI absorption. However, no clinically relevant effect on overall absorption of tested oral medicinal products (paracetamol, oral contraceptives, atorvastatin, digoxin, metformin or warfarin) was observed with semaglutide 2.4 mg (weekly), the maximal dose that we are planning in the current study (55). To the best of our knowledge, no specific clinical guidance has been developed on INR monitoring for concomitant coumarin use since the marketing authorization in January 2022. Therefore, management of potential interactions, including INR monitoring in patients receiving coumarin derivatives or drug monitoring for drugs with a narrow therapeutic window, will be conducted according to routine clinical practice and the treating physician's judgment.

As for all medications, participants are at risk of developing allergic reactions, including serious reactions such as angioedema and anaphylaxis. Participants with known or suspected hypersensitivity to semaglutide

or related GLP-1 RA will be excluded from the study (55). Participants will be informed about the characteristic symptoms of serious hypersensitivity reactions and instructed to discontinue semaglutide and contact their health care provider immediately if symptoms suggestive of a systemic hypersensitivity reaction occur.

Acute pancreatitis has been observed with GLP-1 receptor agonists (55). Participants with a history of chronic pancreatitis or recent acute pancreatitis will be excluded from participation. Participants will be informed about characteristic symptoms of acute pancreatitis, including severe and persistent abdominal pain, possibly radiating to the back, with or without vomiting. Further, in the case of suspicion of pancreatitis, semaglutide will be discontinued. If pancreatitis is confirmed, semaglutide will not be restarted.

In some studies, with overweight patients without T2D, the risks of cholelithiasis or cholecystitis are marginally higher compared to placebo (55). In other studies, however, no increased risk of gallbladder-related disorders or cholelithiasis have been found. Gallbladder-related events, including cholelithiasis or cholecystitis, may also occur in association with weight loss and GLP-1 RA treatment. Symptoms suggestive of gallbladder disease will be evaluated clinically and appropriate clinical follow-up will be initiated if indicated.

As the SmPC states that semaglutide is not recommended in patients with severe hepatic impairment, participants with severe hepatic impairment (Child-Pugh class C) will be excluded from the Trial (55). No dose adjustment is required in patients with mild or moderate hepatic impairment, although clinical experience remains limited. The SmPC states that semaglutide should be used cautiously in patients with mild or moderate hepatic impairment. Therefore, participants with mild or moderate hepatic impairment will be closely followed up throughout the study for adverse events and treatment tolerability.

There is limited clinical experience with semaglutide in patients with inflammatory bowel disease (55) A retrospective cohort study did not demonstrate an increased risk of adverse events associated with semaglutide treatment in participants with inflammatory bowel disease (57) However, as a precaution due to the known GI side-effects of semaglutide, participants with unstable inflammatory bowel disease will be excluded from the Trial. Participants with stable inflammatory bowel disease will be closely followed up throughout the study for GI adverse events and treatment tolerability.

Epidemiological data suggest an increased risk of non-arteritic anterior ischaemic optic neuropathy (NAION) during treatment with semaglutide (55). Participants will be informed about symptoms suggestive of NAION, including sudden vision loss or rapidly worsening eyesight, and instructed to seek immediate medical attention if such symptoms occur. Ophthalmological evaluation will be performed in participants presenting with sudden vision loss or rapidly worsening eyesight and semaglutide treatment will be interrupted pending evaluation and discontinued if NAION is confirmed.

Participants will be informed that discontinuation of semaglutide treatment may be associated with partial weight regain and reversal of some cardiometabolic benefits, as observed following discontinuation of GLP-1 receptor agonist therapy (58). To mitigate this risk, participants will continue routine follow-up within the multidisciplinary obesity clinic of UZ Leuven according to standard clinical care pathways during and after the trial. Participants may, based on their clinical response and treatment indication, continue semaglutide therapy, transition to an alternative pharmacological obesity treatment, and/or receive ongoing multidisciplinary lifestyle and behavioral support, in accordance with routine clinical practice. Participants will be informed that continuation of semaglutide treatment after trial completion may involve personal financial costs depending on the reimbursement status in Belgium.

Risks regarding study assessments

Positron Emission Tomography (PET) scanning involves radiation exposure, but risks are minimal. The maximum administered dose will be determined by medical radiation physics, and in previous studies, a dose of 225 MBq was used, corresponding to a radiation burden of 4.725 mSv. This falls within the WHO IIb classification (1–10 mSv) and poses no acute or chronic safety hazard. Furthermore, participants cannot

take part in PET scanning if they have been exposed to a significant amount of ionizing radiation within the last twelve months.

Magnetic Resonance Imaging (MRI) scanning is a very safe imaging technique as it does not involve ionizing radiation. The primary consideration is the strong magnetic field of the scanner; therefore, participants with contraindications such as pacemakers, implants, unremovable jewelry, metallic foreign bodies, or claustrophobia will not be included. Additionally, multiple demagnetizing checkpoints will be implemented before entering the scanning room to maximize safety. To minimize noise during scanning, we will give earplugs and headphones to each participant. The images we will obtain through the MRI scanning protocol are experimental procedures dedicated to studying particular features of brain structure and function. The study images are in many cases unsuited for detection of abnormalities, should these be present. It is therefore possible, even if the images are reviewed by qualified radiologists, which is standard procedure, that an abnormality is undetected. In case of an incidental finding, a physician of the team, Dr. Roman Vangoitsenhoven, will contact the participant's treating physician and/or general practitioner, who will then inform the participant. We will not scan participants refusing the reporting of (incidental) findings on brain images.

For PET imaging and Mixed Meal Tolerance Test (MMTT) visits, a catheter will be placed for blood collection. Intravenous and arterial catheter placement is a common and routine procedure in healthcare. When placed properly and in short durations (<72 hours) by competent personnel, complications are mostly mild, ranging from hematoma (bruising), minor haemorrhage (bleeding) and local extravasation (swelling). However, in a small number of people, placement of needles/catheters/blood collection might trigger a vasovagal reaction, which can cause a brief drop in blood pressure and fainting. To minimise the risk of fainting, placement of the catheter will be done on a reclining chair or bed.

The preservative in the 24h urine collection container contains 18% hydrochloric acid. Accidental direct contact can cause burns, and inhalation can cause respiratory irritation. In case of contact, rinse immediately with plenty of water and seek medical advice. Safety instructions are also included when you take the urine container with you. 24h urine collection is frequently performed in routine clinical care and is safe when instructions are followed.

Completing Experience Sampling Methodology (ESM) questionnaires could be burdensome and time-consuming for participants and could interfere with their regular daily life. The questionnaires are intentionally kept short and while participants are encouraged to respond to as many beeps as possible, they are told to refrain from responding if they are in a situation where attending to their mobile device is considered dangerous, e.g. whilst steering a vehicle or using machinery. During daily diaries, it is possible that participants will experience discomfort upon being confronted with negative emotions and eating behavior that they are required to report in this study, but the literature does not appear to support the notion that this is an issue (59,60). The study could also be beneficial for participants, as novel therapies include daily diaries to improve emotional awareness and self-management.

The use of wearables brings no risks for the participants. In a minority of cases, participants have mild skin irritation when wearing a wristband. In case this causes significant discomfort, the participant will be advised to remove the wearable for the remainder of the study. Further, wearables will operate in a blinded display mode that shows only the time and battery level, which prevents participants from viewing their own data and thereby eliminates the chance of reactivity.

Urine and hair sample collection, as well as filling in questionnaires, pose no or negligible risks.

Risks regarding pregnancy and breastfeeding

Semaglutide should not be used during pregnancy. Animal studies have shown reproductive toxicity, while available human pregnancy data are limited. In addition, PET imaging procedures involving radiotracers may expose the fetus to ionizing radiation. Pregnant or breastfeeding women, women intending to become pregnant during the trial or within 2 months after the last semaglutide dose and WOCBP not using a highly effective contraceptive methods will be excluded (cf. 3.1. Inclusion and exclusion criteria). WOCBP may only participate after a negative pregnancy test at screening. Pregnancy testing will be performed monthly during the trial and before PET imaging visits. Additional testing will be performed if pregnancy is suspected. We will conduct the measurements in compliance with the protocol, good clinical practice (GCP), and radiation protection (Federal Agency for Nuclear Control (FANC)) regulations.

Benefits regarding treatment with semaglutide

Semaglutide has been shown to reduce body weight and BMI, as well as decrease self-reported BE scores, highlighting its beneficial effects on both metabolic and behavioral outcomes (61). In addition, the SELECT Trial demonstrated that semaglutide has significant cardiovascular benefits in patients with obesity, including a reduced risk of major adverse cardiovascular events and improved overall survival, further supporting its favorable safety profile (62)

2 Trial Objectives and Design

2.1 Trial objectives

This project aims to establish a sound scientific foundation for optimizing patient selection for GLP-1-based pharmacotherapy as a treatment for obesity across the BE spectrum, as well as to identify its mechanisms of action. We will investigate semaglutide's impact on three motivational psychobiological processes driving eating behavior and weight regulation: i) homeostatic hunger/satiety signaling (appetite-related sensations, metabolic gut-brain hormones), ii) reward processing (food liking, wanting, craving) and its dopaminergic neurochemical basis, and iii) emotional state (stress, affect, negative urgency). Hereto, we will combine advanced multimodal neuroimaging with biochemical, behavioral, and psychological measurements, both lab-based and in daily life. Specifically, we aim to build a multivariate predictive model for treatment efficacy based on a combination of these measurements to identify the best candidates for semaglutide treatment. Finally, by identifying semaglutide's mechanism of action, we aim to identify key treatment targets to further improve treatment of obesity across the BE spectrum, and more broadly, disorders of reward system function, including mood disorders, EDs and addiction. Specifically, we aim to address the following objectives:

Objective 1: Identify for whom semaglutide works best, i.e., to predict heterogeneity of treatment response based on baseline characteristics. Specifically, we aim to determine whether the baseline BES score predicts the change in BMI after semaglutide treatment and whether other baseline eating behavior characteristics (excluding the BES score) predict the change in BES score after semaglutide treatment. Therefore, Aim 1.3 constitutes the primary objective, while Aims 1.1 and 1.2 provide the necessary descriptive analyses to address Aim 1.3.

- Aim 1.1.: To conduct deep phenotyping of patients in terms of eating behavior/BE, using questionnaires and ESM. (secondary endpoint: eating behavior)
    - Hypothesis 1.1.: Large between-subject variability on all eating behavior dimensions.

- Aim 1.2.: To investigate the effect of semaglutide on anthropometric measures and BE severity. (primary endpoints: change in BMI and change in BES score; secondary endpoints: change in waist/hip ratio, change in body composition)
    - Hypothesis 1.2.: Semaglutide will be highly efficacious on all anthropometric measures, with large effect sizes, and it will also reduce BE severity with a medium effect size.

- Aim 1.3.: To investigate if baseline inter-individual differences in eating behaviour/BE moderate the effects in Aim 1.2. (primary endpoints: change in BMI, change in BES score; secondary endpoints: change in waist/hip ratio, change in body composition, eating behavior)
    - Given the lack of previous research, we refrain from formulating specific hypotheses about the moderation effects' significance, direction or magnitude.

Objective 2: Investigate how semaglutide works.

<u>Eating behavior:</u>

- Aim 2.1.: To investigate the effect of semaglutide on eating behavior through questionnaires. (secondary endpoint: eating behavior)
- Aim 2.2.: To investigate the effect of semaglutide on daily-life eating behavior as assessed through ESM. (secondary endpoint: eating behavior)

Gut-brain hunger and satiety signaling:

- Aim 2.3.: To investigate the effect of semaglutide on peripheral DA signaling in plasma and urine, plasma levels of metabolic hormones and self-report appetite-related sensations during a mixed-meal test. (secondary endpoints: gut hormone levels, blood and urinary DA levels, appetite-related sensations)
- Aim 2.4.: To investigate the effect of semaglutide on self-report appetite-related sensations in daily life assessed through ESM. (secondary endpoints: appetite-related sensations)

Reward processing:

- Aim 2.5.: To investigate the effect of semaglutide on neural responses to a food reward task. (secondary endpoint: neural responses to food reward)
- Aim 2.6.: To investigate the effect of semaglutide on food-induced DA release in a subset of patients with (i) no/minimal BE and (ii) severe BE. (secondary endpoint: DA release in the brain in response to food reward)
- Aim 2.7.: To investigate the effect of semaglutide on self-report measures of food liking, wanting and craving as assessed through ESM in daily life and through questionnaires in the laboratory. (secondary endpoint: food wanting, liking and craving)

Emotional state:

- Aim 2.8.: To investigate the effect of semaglutide on self-reported emotional state in the laboratory. (secondary endpoint: emotional state (stress, positive affect, negative affect and negative urgency))
- Aim 2.9.: To investigate the effect of semaglutide on daily-life stress, positive and negative affect, and negative urgency, as measured using ESM, and on autonomic nervous system (ANS) activity/responses as measured by wearables. (secondary endpoints: emotional state (stress, positive affect, negative affect and negative urgency), heart rate, heart rate variability)
- Aim 2.10.: To investigate the effect of semaglutide on biological markers of chronic stress, i.e., hair cortisol levels, serum SCFA levels, serum cytokine levels, blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BSCFB) integrity/permeability and neuroinflammation. (secondary endpoints: chronic hair cortisol levels, SCFA blood levels, cytokine blood levels, BBB/BSCFB integrity/permeability)

Hypothesis 2.1.-2.10.: The effects of semaglutide on anthropometric measures and BE severity will be mediated by several measures of eating behavior, as well as one or more of the three motivational psychobiological processes underlying eating behavior. The current literature does not allow to formulate specific hypotheses about their relative contribution.

Objective 3: Examine whether semaglutide's psychobiological mechanisms of action differ along the BE spectrum.

- Aim 3.1.: To investigate if the psychobiological mechanisms underlying the effect of semaglutide on anthropometric measures differ along the BE spectrum. (primary endpoints: change in BMI; secondary endpoints: change in waist/hip ratio and change in body composition)
    - Hypothesis 3.1.: Reward and emotional state processes play a more important role relative to homeostatic processes in patients who score higher on BE severity.

Objective 4: Develop a multivariate prediction model for successful weight and BE severity reduction in patients with obesity across the BE spectrum.

- Aim 4.1.: To investigate whether the amount of weight and BE severity reduction can be accurately predicted from baseline data on eating behavior and its underlying psychobiological processes. (primary endpoints: change in BMI and change in BES score; secondary endpoints: change in waist/hip ratio and change in body composition)
    - No specific hypotheses are formulated on the accuracy of prediction or the relative contribution of the different processes.

2.2 Primary Endpoints

- Change in BMI after semaglutide treatment (baseline vs at 6 months of semaglutide treatment)
- Change in Binge Eating Scale (BES) score after semaglutide treatment (baseline vs at 6 months of semaglutide treatment)

2.3 Secondary Endpoints

Secondary endpoints in the narrow sense

- Change in waist/hip ratio after semaglutide treatment (baseline vs at 6 months of semaglutide treatment)
- Change in body composition after semaglutide treatment (baseline vs at 6 months of semaglutide treatment)

Mechanistic secondary endpoints (mediators)

Additionally, we will determine the mediating and/or moderating factors that potentially underlie semaglutide-induced changes in BMI and BES, including changes in the following after semaglutide treatment (baseline vs at 6 months of semaglutide treatment):

- Gut hormone blood levels
- Blood and urinary DA levels
- Appetite-related sensations
- DA release in the brain in response to food reward
- Neural responses to food reward
- Food liking, wanting and craving
- Emotional state (stress, positive affect, negative affect and negative urgency)
- Eating behavior
- Heart rate (HR) and heart rate variability (HRV)
- Blood pressure (BP)
- Chronic hair cortisol levels
- SCFA blood levels
- Cytokine blood levels
- BBB/BSCFB integrity/permeability
- Neuroinflammation

Mechanistic secondary endpoints (mediators) are included to assess the effect of treatment on intermediate variables. This is necessary to enable mediation analyses (objective 2), as evidence of an effect of treatment on the mediator is a prerequisite for evaluating whether changes in the mediator contribute to the effect on the primary endpoints.

2.4 Trial Design

This study is a prospective, single-center, longitudinal, interventional study in individuals with obesity across the BE spectrum starting semaglutide treatment, to assess semaglutide's effect on psychobiological drivers underlying eating behavior. Upon recruitment, participants (n=96) will be stratified into three groups (n=32 each) based on their BES score: low (< 17), intermediate (18-26) and high (>27).

Measurements will be collected at the baseline visits (T0), at 3 months of treatment (T3) and at 6 months of treatment (T6). The duration of a 6-month treatment period is chosen as this timepoint is more stable in terms of body weight and eating behavior for most patients compared to 3 months post-therapy, while maintaining feasibility within the duration of the project (63).

In addition to routine care data, blood sample data, urinary sample data, hair sample data, questionnaire data, and fMRI data will be collected at T0 and T6. Furthermore, a subsample of 30 participants from both the low and high BES groups (matched for age, sex and BMI) (*n*=64) will undergo PET scanning at T0 and T6 as well. Throughout the Trial participants will undergo ESM at three bursts (T0, T3 and T6), each lasting three weeks, with data collected only on Thursdays, Fridays and Saturdays. During each burst, Garmin wearables will continuously measure HR and HRV. In total, there will be two study visits, namely a baseline (T0) MRI-MMTT visit and an MRI-MMTT visit at 6 months of treatment. A subsample of 64 participants will have two additional visits, namely a baseline PET scan visit and a PET scan visit at 6 months of treatment. Furthermore, during the preparation day (1 day before each study visit), participants will be asked to refrain from alcohol, caffeine, and strenuous exercise.

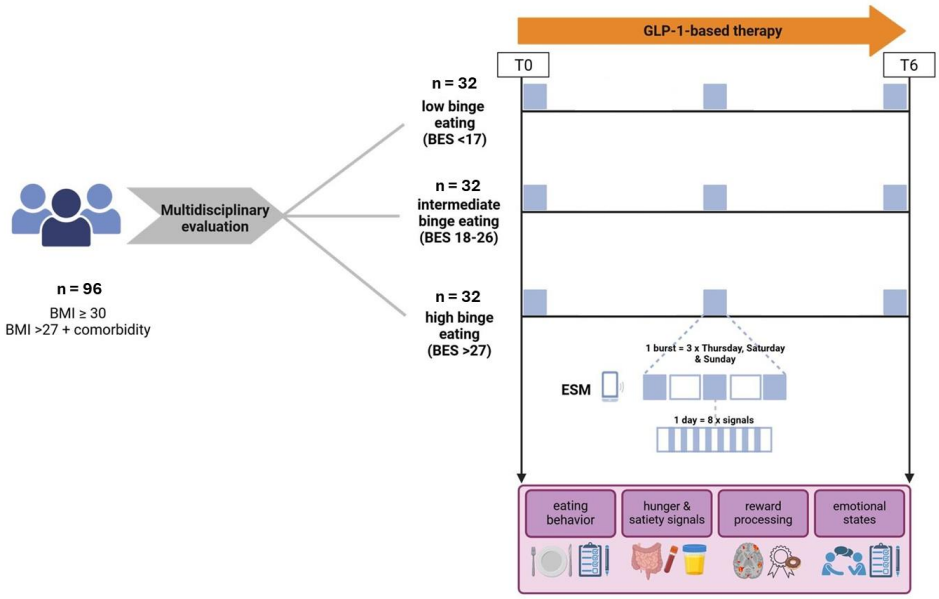


Figure 1. Overview of the study design

2.4.1 Use of In-Vitro Diagnostic (IVD) or Medical Device (MD)

This Trial is not investigating any IVD or MD.

2.5 Expected Duration of the Trial

The expected Trial duration for a single participant will be between approximately 7 months, depending on scanner availability. The full Trial duration is expected to be 3,5 years (including subject recruitment, data collection and data analysis), while data collection (starting from the first participant visit to the last participant visit) is expected to be 2,5 years, as shown in the table below:

Trial timeline	2026				2027				2028				2029			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Subject recruitment																
Data collection																
Data analysis																
Result writing and publication																

The End of Trial is defined as Last Patient Last Visit (LPLV). The CI shall notify the CA/EC of the end of the Trial using the CTIS portal. The CI will submit a final report with the results of the study, including any publications/abstracts, to the CA/EC within 1 year of Trial termination. The CTIS portal will be used for communication with the CA/EC of all participating Member States.

The Sponsor, CA/EC can decide to halt or prematurely terminate the Trial when new information becomes available whereby the rights, safety and well-being of Trial participants can no longer be assured, when the integrity of the Trial has been compromised, or when the scientific value of the Trial becomes obsolete and/or unjustifiable.

Circumstances requiring premature treatment interruption or discontinuation of the Trial, include but are not limited to:

- Safety concerns related to IMP or unacceptable intolerability (including BMI < 18.5 kg/m^2)
- Trial participation while in violation of the inclusion and/or exclusion criteria
- Pregnancy
- Intention of becoming pregnant during the Trial and within 2 months after the last semaglutide dose
- Participation withdrawal
- Missing 4 consecutive doses of the IMP

Should the Trial be temporarily suspended or, ended prematurely, the CI will notify the CA/EC and include the reasons for suspension/premature termination within 15 days of the decision.

3 Trial Population / Eligibility Criteria

3.1 Inclusion criteria

Participants eligible for inclusion in this Trial must meet **all** of the following criteria:

1. Voluntary written informed consent of the participant or their legally authorized representative has been obtained prior to any screening procedures
2. At least 18 years of age and under 65 years of age at the time of signing the informed consent form (ICF)
3. BMI ≥ 30 kg/m² or BMI ≥ 27 kg/m² with at least one weight-related comorbidity
4. WOCBP* must have a negative urine pregnancy test at screening and agree to undergo monthly pregnancy testing throughout the study.
5. WOCBP must agree to use a highly effective contraceptive methods (as discussed below**), defined as a method with a failure rate of <1% per year when used consistently and correctly, from screening until at least 2 months after the last dose of semaglutide to account for the prolonged elimination half-life and relevant systemic exposure of semaglutide.

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming postmenopausal unless permanently sterile.

Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as amenorrhoea for at least 12 consecutive months without an alternative medical cause in a female >45 years of age. Females ≥60 years of age can be considered postmenopausal. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm postmenopausal status in women not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient. Women receiving HRT whose menopausal status is uncertain will be considered WOCBP. For females with permanent infertility due to an alternate medical cause other than those listed above, investigator discretion should be applied in determining eligibility.

***** Contraception requirements: highly effective methods (Failure rate of <1% per year when used consistently and correctly) include:***

- *Combined (oestrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation:*
  - *oral*
  - *intravaginal*
  - *transdermal*
- *Progestogen-only hormonal contraception associated with inhibition of ovulation:*
  - *oral*
  - *injectable*
  - *implantable*
- *Intrauterine device (IUD)*
- *Intrauterine hormone-releasing system (IUS)*
- *Bilateral tubal occlusion/ligation*
- *A vasectomised partner, provided the vasectomised partner is the sole sexual partner of the WOCBP and absence of sperm has been medically confirmed*
- *Complete sexual abstinence from heterosexual intercourse during the entire period of risk associated with semaglutide treatment.*
- *Same-sex partner(s) only, provided this is the WOCBP's exclusive sexual practice during the Trial period and for at least 2 months after the last dose of semaglutide*

The use of condoms by the partner of the WOCBP is only allowed as an add-on to a highly effective contraceptive method listed above. WOCBP using oral hormonal contraception will be advised that GI adverse events (e.g., vomiting) associated with semaglutide may reduce contraceptive effectiveness. In such cases, WOCBP should temporarily use an additional barrier method or an alternative highly effective contraceptive method should be considered.

No contraceptive methods are required for male participants, as clinically relevant embryo-fetal exposure via seminal transfer of semaglutide is considered unlikely. In addition, WOCBP must also refrain from oocyte donation during treatment and until at least 2 months after the final dose of semaglutide.

All participants who are considered for Trial participation, per the above criteria will be documented via applicable log forms in Investigator Site File (including Screen Failures).

3.2 Exclusion criteria

Participants eligible for this Trial must **not** meet any of the following criteria:

1. Any form of diabetes mellitus (T1- or T2DM), defined as current hemoglobin A1c (HbA1c) > 6.5% or the use of antihyperglycemic drugs (metformin is permitted if prescribed for an indication other than T2DM and at a stable dose for at least 3 months days prior to screening)
2. Use of incretin-based drugs, including GLP-1RAs (including dual agonists) and dipeptidyl peptidase-4 (DPP-4) inhibitors or amylin analogues within 3 months prior to screening
3. Contraindications for semaglutide: history of pancreatitis, medullary thyroid carcinoma or multiple endocrine neoplasia type 2 (MEN2)
4. Known hypersensitivity to the active substance semaglutide or to any of the excipients of Wegovy
5. Known clinically significant gastric emptying abnormality such as severe gastroparesis or gastric outlet obstruction
6. Unstable inflammatory bowel disease without any changes in medication within 3 months prior to screening
7. Chronic kidney disease 3b or worse, defined as estimated Glomerular Filtration Rate (eGFR) < 45 ml/min/1.73m²
8. Severe hepatic impairment (Child-Pugh class C)
9. Chronic heart failure classified as being in New York Heart Association (NYHA) Class IV at screening

10. Major surgery scheduled during the duration of the study
11. Previous bariatric surgery
12. Disorders known to affect DA signaling significantly, including movement disorders such as Parkinson's disease, prolactinoma, paraganglioma and pheochromocytoma
13. Active use of medication affecting DA signaling, including antiparkinson medications, attention deficit hyperactivity disorder (ADHD) medications, antipsychotics, naltrexone-bupropion for weight management or antidepressants; use of serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), or trazodone is permitted if participants are on a stable dose for at least three months. Incidental use of antiemetics or sedative hypnotics is allowed but not within 48 hours prior to a study visit.
14. Current or recent (within the past 6 months) clinically significant alcohol or drug misuse (excluding nicotine) that, in the investigator's judgment, may compromise participant safety or the integrity of study data.
15. Active severe neuropsychiatric disorders, including dementia, psychotic disorder, bipolar disorder and severe depression
16. Acute suicidality or history of suicide attempt within 5 years prior to screening
17. Any condition or major illness which, in the investigator's opinion, may jeopardise participant safety or compliance with the protocol
18. Unable to understand the risks, realistic benefits and compliance requirements of each program
19. Unwillingness to be informed about incidental findings that may be important for the participant's health
20. Recent participation (< 30 days) or simultaneous participation in an interventional Trial with an investigational medicinal product (IMP) or device
21. Women who are currently pregnant, breastfeeding or plan to become pregnant during the Trial or within 2 months after the last semaglutide dose, or of childbearing potential and are not using highly effective contraceptive methods (cf. inclusion criteria regarding contraceptive methods).
22. Imaging-related exclusion criteria:
    - For PET scanning (subset *n*=64): exposure to a significant amount (> 1mSv) of ionizing radiation in other research studies within the last 12 months
    - For MRI scanning: any condition that would interfere with MRI scanning (e.g., cochlear implant, metal fragments in the eyes, cardiac pacemaker, neural stimulator and metallic body inclusion or other metallic implants in the body which may interfere with MRI scanning)
    - Claustrophobia or too much uneasiness in limited spaces (to tolerate confinement during scanning procedures)
    - Severe back problems, interfering with lying in supine position in the scanner without movement for long durations

Participants who meet one or more of the above exclusion criteria **must not proceed** to be enrolled/randomized in the Trial and will be identified via applicable log forms in Investigator Site File.

4 Trial Procedures

4.1 Selection of Participants / Recruitment

Potential participants will be identified and recruited during patient consultations at the UZ Leuven Obesity Clinic and at the UPC KU Leuven Eating Disorders Outpatient Clinic. The study will also be presented using a recruitment flyer (Appendix 3). Participants will be recruited via flyers spread around the university buildings of KU Leuven and within UZ Leuven hospital. The study advertisement will also be displayed on digital screens across UZ Leuven. Additionally, the advertisement will be posted on the hospital's intranet under "prikbord" and shared via the platforms of ED peer support organisations.

4.2 Trial assessments and procedures

Trial procedures and their timing are summarized in the Trial Flowchart.

4.2.1 Participant consent and withdrawal of consent

The Trial will be conducted only on the basis of prior informed consent by the Trial participants and/or their legally authorized representative(s). As such, no Trial-related procedures will be conducted prior to obtaining written informed consent from potential Trial participants.

The process for obtaining and documenting initial and continued informed consent from potential Trial participants will be conducted in accordance with GCP, applicable regulatory requirements and internal Standard Operating Procedures (SOPs).

All originally signed obtained ICFs must be retained/archived in the Investigator Site File (ISF) at the Participating Site and must not be destroyed (even when a scanned copy is available) before expiration of the legal archiving term as defined in the protocol section entitled "Archiving".

Participants may voluntarily withdraw consent to participate in the Trial for any reason at any time. The participant's request to withdraw from the Trial must always be respected without prejudice or consequence to further treatment. Consent withdrawal will be documented in the participant's medical record.

Trial data and samples collected before withdrawal can be used in the Trial. No new Trial data or samples will be collected after withdrawal of the participant.

4.2.2 Randomization Procedure / Blinding (if applicable)

Not applicable

4.2.3 Unblinding (if applicable)

Not applicable

4.2.4 Other Trial assessments and procedures

4.2.4.1 Screening and ICF procedure

As part of the standard clinical intake process at the Obesity Clinic, patients will complete the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 (GAD-7) to evaluate the presence and extent of depressive and anxiety symptoms, respectively. Further, as part of the clinic's standard multidisciplinary consultation, patient eligibility for semaglutide treatment will be evaluated.

Patients who are eligible for semaglutide treatment and meet the initial in- and exclusion criteria will be approached during an appointment at the clinic by a member of the study team who will provide a detailed explanation of the study. For patients interested in participating, the informed consent process will be conducted, and written consent will be obtained.

After this, additional screening steps will be conducted. Participants will first complete the BES to evaluate the presence and extent of binge eating, as reflected by the BES score. If the participant has not had a routine blood panel in the 4 months prior to screening, a blood sample will be taken to screen for any form of diabetes and chronic kidney disease 3b or worse. If the participant has had a routine blood panel in the 4 months prior to screening, the results of this blood panel will be used for screening. Details of the routine blood panel tests performed by the UZ Leuven can be found in Appendix 4. Further, a urine drug test will be performed to screen for drug use and the Mini International Neuropsychiatric Interview Simplified (MINI-S) will be administered to screen for psychiatric disorders and drug (ab)use. Lastly, if participants remain eligible after these last screening steps, we will first guide them in installing and setting up the mPath app on their personal smartphones. Afterward, they will receive training on completing the ESM protocol in mPath. Additionally, participants will receive a wearable device that will be set up during this visit as well.

4.2.4.2 Stratification

Participants will be stratified according to a low (< 17), intermediate (18-26) or high (>27) BES score (*n=32* each) to achieve a total sample size of *n=96* with a Gaussian distribution for BES score, which will be used in statistical analyses as a continuous rather than ordinal (i.e. binned based on cutoffs) variable to maximally preserve true variability and statistical power. The frequency sampling method will be employed to match the three groups for age, sex and BMI, thereby achieving a similar distribution for these three variables across the BES continuum, effectively avoiding confounding.

4.2.4.3 Laboratory assessment of gut-brain hunger and satiety signaling

Mixed Meal Tolerance Test (MMTT)

To investigate how semaglutide modulates gut-brain signaling related to hunger and satiety, participants will undergo an MMTT at T0 (baseline) and T6 (at 6 months of treatment). The MMTT allows us to assess metabolic and hormonal responses to a standardized mixed nutrient meal and to determine how these responses change following semaglutide treatment.

Participants will undergo an MMTT after the MRI scan. A venous catheter will be inserted into a forearm vein to facilitate repeated sampling, with approximately 25 mL of blood collected at each time point. A first blood sample will be collected at baseline, prior to ingestion of the mixed meal. Participants will then consume 200 ml of Ensure Plus (300 kcal; 9.84 g fat, 40.4 g carbohydrates, 12.5 g protein; Abbott Laboratories), after which additional blood samples will be obtained at fixed intervals over a 2-hour period (t+00, t+15, t+30, t+60, t+90 and t+120 min).

Measurements will include glucose, insulin, motilin, GLP-1, gastric inhibitory polypeptide (GIP), leptin, (active and total) ghrelin, peptide YY (PYY) and DA. Plasma DA concentrations will be quantified using liquid chromatography-tandem mass spectrometry (LC–MS/MS) with isotope dilution. Circulating concentrations of insulin, motilin, GLP-1, GIP, leptin, ghrelin and PYY will be measured using standard enzyme-linked immunosorbent assays (ELISAs) or specialized radioimmunoassays (RIAs) developed at our department (already established collaboration with [redacted]). Glucose levels will be determined using a glucometer.

Additionally, for the baseline blood sample, SCFA and cytokine levels will be measured. Serum SCFAs will be quantified by gas chromatography-mass spectrometry (GC-MS) after derivatization with 2,4-difluoroanilin and extraction into ethyl acetate (collaboration [redacted]). Serum cytokines will be measured using an electrochemoluminescence multiarray system (collaboration [redacted]). IL-6 and TNF-α are of primary interest, other cytokines in the panel (V-PLEX human biomarker 40-plex kit, MSD) are exploratory (64). High-sensitive CRP will be measured in the UZ Leuven Central Lab using a latex-enhanced immunonephelometric test.

At each blood sampling time point (baseline, t+00, t+15, t+30, t+60, t+90 and t+120 min), we will measure HR and BP with a blood pressure monitor, and participants will also complete Visual Analogue Scales (VAS) to asses self-reported ratings of hunger, appetite, fullness, satiety, prospective food consumption, and nausea. Each VAS will consist of a 100-mm horizontal line anchored by “not at all” (0) and “extremely” (100), accompanied by a specific question. See Appendix 5 for the specific VAS questions.

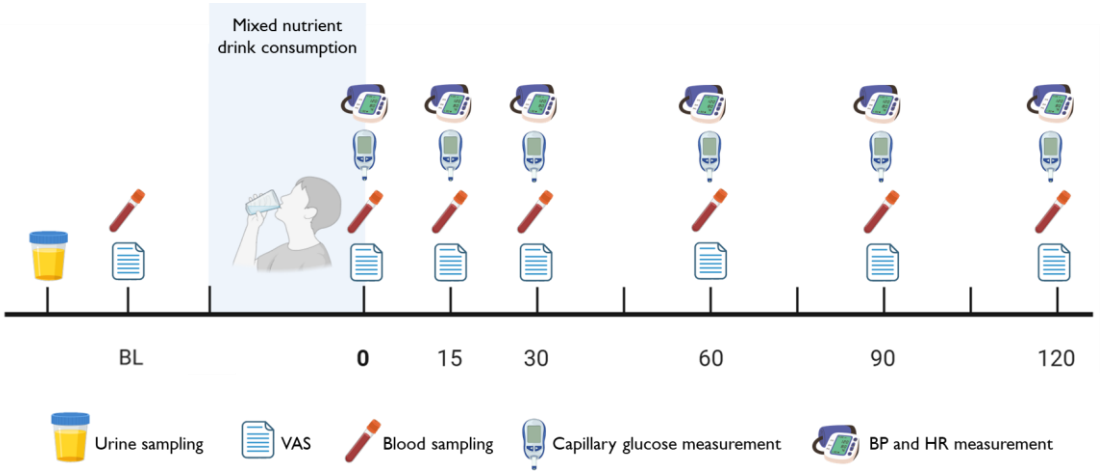


Figure 2. Overview of the Mixed Meal Tolerance Test (MMTT)

24h urine analysis

On the day before each MMTT visit at T0 (baseline) and T6 (at 6 months of treatment), participants will collect urine over 24h, preserved with hydrochloric acid, for the analysis of DA and c-peptide. DA concentration will be measured using LC-MS/MS with isotope dilution ([redacted]). C-peptide will be measured using ELISAs. This will provide insight into the daily DA and insulin production, complementing data from the fasted state and MMTT.

4.2.4.4 Laboratory assessment of reward processing through brain imaging

Functional MRI (fMRI) during the Food Incentive Delay (FID) task

To investigate semaglutide's effect on brain activity related to anticipatory and consummatory food reward, participants will undergo the Food Incentive Delay (FID) task during fMRI scanning at T0 (baseline) and T6 (at 6 months of treatment). The scanner-adapted FID task is designed to probe neural responses associated with the anticipation (cue and delay phases) and receipt of food rewards (feedback phase), thereby providing insight into the central mechanisms through which semaglutide influences food-related reward circuitry underlying eating behavior. Each imaging scan will start with the acquisition of high-resolution structural T1-weighted images for anatomical localization, whereafter task-based functional sequences will be run while participants undergo the FID task. All fMRI sessions will take place in the morning after an overnight fast of at least 10 hours at the Department of Radiology of UZ Leuven using the 3 Tesla Siemens Magnetom Cima.X scanner exclusively dedicated to neuroimaging research (operational July 1st 2026). The evening before each fMRI visit, participants will consume a standardized meal.

The FID task is an adaptation of the Monetary Incentive Delay (MID) task, modified to engage food-related motivational and reward processes, which allows an efficient probing of both anticipation and consumption of reward (65). During scanning, participants can earn "snack points" (SP), which they can exchange for pastries at the end of the testday, enhancing ecological validity and motivation.

The task will consist of 2 identical blocks, divided into 2 imaging runs. Each block will consist of 55 Trials each. During each Trial, SP will be able to be gained. The degree of potential reward will vary between 3 levels as indicated via graphical cues. Each Trial will start with the presentation of a symbol ('cue') for 750 msec, indicating the number of SPs subjects will be able to win during this Trial. Cues will consist of an empty circle (0 SP), a circle with one line (2 SP), or a circle containing two lines (10 SP). After an anticipation period ('delay') of 3000 msec, subjects will have to correctly react to one out of two different symbols ('target', i.e. a triangle inclined to the right or to the left). Using an MR-compatible button box, subjects will

answer with a left or right button press, corresponding to the direction of the target within a fixed interval of 1000 msec.

To ensure a steady rate of reward and non-reward throughout all subjects, a probabilistic reward pattern will be applied, i.e. reward will not be paid out in 15 predefined Trials out of 55 Trials. Immediately after target presentation, feedback will appear ('feedback') for 1500 msec, notifying subjects about the amount of SPs they will have won during the Trial and about their cumulative total. For this purpose, images of either an empty basket (0 SP won), one croissant (2 SP won), or a collection of croissants (10 SP won) will be shown.

Intertrial intervals (ITI) will be jittered, ranging from 1 to 8 sec (mean ITI = 3.5 sec). An incorrect button press will result in a zero pay-out. If a button press is missed, a penalty of -10 SP will be applied to ensure that subjects will pay attention to every Trial. The maximum number of snack points that participants will be able to win during 1 run will be 150 SP, with every pastry being worth 50 SP.

At the start of the study visit, subjects will perform a try-out version of the task for 3 min, to avoid learning effects, while the task will be performed during scanning. They will not receive any SP during this practice session.

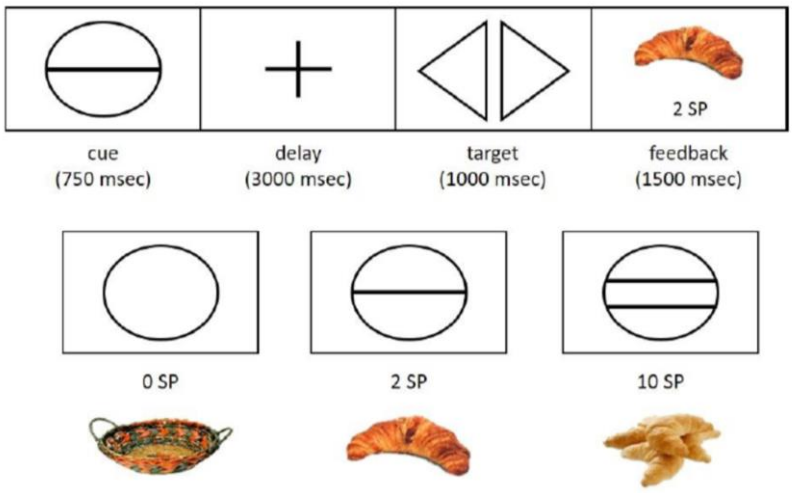


Figure 3. The Food Incentive Delay (FID) task

[18^F]-fallypride positron emission tomography (PET) imaging

To assess semaglutide's effect on food-induced DA release in both striatal and extrastriatal regions of the brain reward system, PET imaging will be performed using the high-affinity D_2 receptor (D_2R) antagonist, [^{18}F]-fallypride, as a radiotracer (66). Our lab has extensive experience with this radiotracer. A subset (*n*=64) of 32 patients from both the low and high BES groups (matched for age, sex and BMI) will undergo this consummatory reward task during PET scanning at T0 (baseline) and T6 (at 6 months of treatment). All [^{18}F]-fallypride PET scans will be performed at the Department of Nuclear Medicine of the University Hospital Leuven using the ultra-high-performance NeuroEXPLORER PET/CT scanner (in collaboration with [redacted]).

PET scans will take place in the morning, between 9 AM and 1 PM, or in the afternoon, between 1:30 PM and 5:30 PM. For the morning sessions, participants will be asked to consume their regular meals, but to refrain from eating or drinking (except water) for at least 12 hours (equal to an overnight fast) immediately preceding their visit. For the afternoon sessions, participants will be asked to consume a light standardized

breakfast between 8 AM and 8:30 AM, and to refrain from eating or drinking (except water) between breakfast and onset of their visit.

Prior to scanning, a venous catheter will be inserted into a forearm vein to be used for slow bolus radioligand injection as well as blood sampling at various time points throughout the study (see further). After an adaptation period of 10 minutes, a first blood sample (= baseline sample)(t-05 min) will be collected and then the actual experimental PET session will start. Patients will be transferred to the scanning room and will be familiarized with the environment. All subsequent procedures will be explained to minimize anticipatory anxiety and/or fear that may affect cerebral blood flow. The tracer will be administered via a single intravenous injection immediately prior to scanning to ensure maximal initial brain uptake. Participants will receive on average 185 MBq (5 mCi) of [^{18}F]-fallypride injected as a slow intravenous 10-second bolus injection. PET dynamic emission scans will be initiated simultaneously upon injection.

Each PET session will consist of four 45-minute acquisition blocks, separated by 15-minute breaks. The first three blocks will capture [^{18}F]-fallypride kinetics during the control condition, while the food reward condition will commence after the third break, approximately 180 minutes post-injection. During the control condition, participants will receive oral deliveries of 8 mL of water every 4 minutes, whereas during the food reward condition, they will receive 8 mL of chocolate milkshake every 4 minutes. During the food reward condition, food images will be presented to enhance responsiveness to the milkshake reward. The milkshake will be prepared using four scoops of Ijsboerke vanilla ice cream, 1.5 cups of 2% milk and two tablespoons of Imperial's chocolate topping. Stimuli will be delivered using a programmable dual syringe pump to ensure precise control of volume, rate, and timing. Two 60 mL syringes, one containing water and the other the chocolate milkshake, will be connected via tubing through a waveguide to a manifold attached to the PET head coil. This procedure has been successfully used in the past in our lab to deliver liquids in the scanner and has been described in detail elsewhere (67).

Blood samples will be collected at various predefined time points, more specifically right before the start of the PET session (t-05 min), during each break (t+45, t+150 and t+165 min), at the start of the reward task (t+180 min) and throughout the reward task (t+195, t+210 and t+225 min). These samples will be used to quantify plasma glucose, insulin, motilin, GLP-1, GIP, leptin, (active and total) ghrelin, PYY and DA levels. Analyses of these metabolites will be performed following the same procedures as described in Section 4.2.4.4.

At predefined time points, participants will complete VAS to assess self-report ratings of appetite-related sensations and food 'liking' and 'wanting'. Each VAS will consist of a 100-mm horizontal line anchored by "not at all" (0) and "extremely" (100), accompanied by a specific question. Appetite-related sensations, including hunger, fullness, satiety and prospective food, will be assessed before and throughout scanning at t-05, t+15, t+45, t+75, t+105, t+135, t+165, t+180, t+195, t+210 and t+225 min. An additional appetite-related sensation, namely appetite, and nausea, will be assessed at t+15, t+75, t+135, t+180, t+195, t+210 and t+225 min. Finally, VAS ratings of 'liking' and 'wanting' will be obtained at t+180, t+195, t+210 and t+225 min. See Appendix 5 for the specific VAS questions.

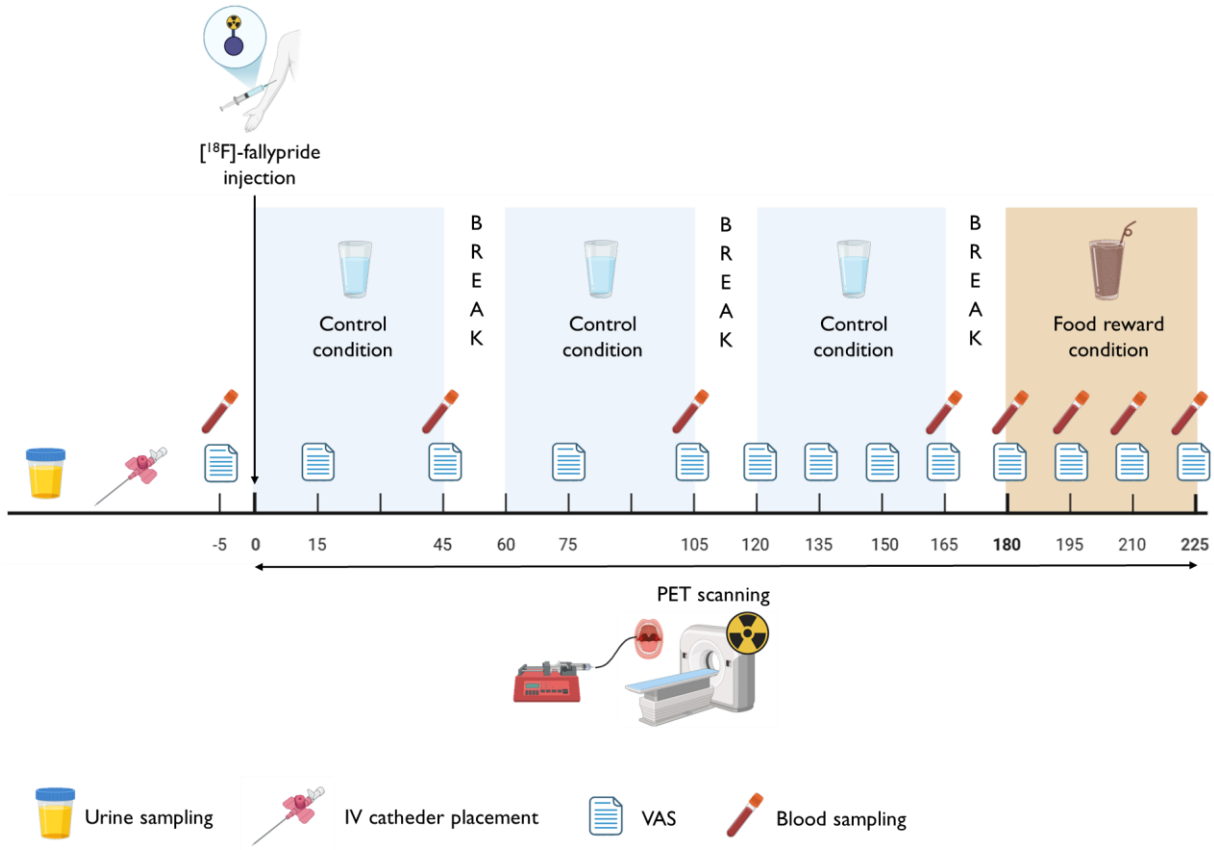


Figure 4. Overview of a PET scanning visit

4.2.4.5 *Laboratory assessment of BBB/BSCFB integrity/permeability*

Multi-echo arterial spin labelling (ASL) (collaboration [redacted]) will be used to calculate the time taken for labelled water molecules to exchange between blood and tissue across the BBB (exchange time) as a proxy measure of BBB integrity/permeability (68). Choroid plexus volume, quantified using high-resolution structural scans, will provide a measure of blood-CSF barrier (BSCFB) permeability. BBB and BCSFB data will be analyzed using FSL and Analyze, respectively (69).

4.2.4.6 *Laboratory assessment of neuroinflammation*

To assess neuroinflammation, we will use an MR spectroscopy PRESS sequence [glial markers myo-inositol (MI) and choline, neuronal metabolism marker N-acetylaspartate (NAA)] (voxels in hypothalamus, ventral striatum, and vmPFC)(70). Data will be analyzed using Osprey (collaboration [redacted]).

4.2.4.7 *Assessment of hair cortisol*

To assess hair cortisol, a lock of hair [length≥ 3 cm (average cortisol over ~3 months), weight ≥ 10 mg] will be collected from the posterior vertex, cut closely to the scalp (71). The wash procedure and steroid extraction will be done using high-performance liquid chromatography-mass spectrometry (71).

4.2.4.8 *Assessment of eating behaviour and emotional state through self-report questionnaires*

On the days of the MRI-MMTT visits, participants will be asked to fill out a battery of questionnaires.

Questionnaires to assess depression and anxiety

The Patient Health Questionnaire (PHQ-9)

The PHQ-9 is a 9-item self-report instrument that screens for depressive symptoms and assesses symptom severity over the preceding two weeks (72). PHQ-9 total scores range from 0 to 27, with lower scores indicating less severe and higher scores indicating greater symptom severity.

The 7-item Generalized Anxiety Disorder Scale (GAD-7)

The GAD-7 is a 7-item self-report scale that screens for GAD and assesses symptom severity, also based on symptoms experienced over the preceding two weeks. Total scores range from 0 to 21, with higher scores indicating greater symptom severity (73).

Questionnaires to assess eating behavior and BE severity

The Binge Eating Scale (BES)

The BES is a 16-item self-report instrument designed to evaluate the presence and behavioural manifestations of BED, as well as the feelings and cognitions surrounding a binge episode (74). The BES will be used to assess BE severity as a continuum. The BES will be used to stratify patients into three groups based on their BES score: low (< 17), intermediate (18-26) or high (> 27). Further, we will use this to assess for whom semaglutide is most efficient, as well as assess if semaglutide affects BE severity.

The BES has not yet been officially translated into Dutch. Therefore, we will translate and culturally adapt the BES in accordance with established international guidelines for questionnaire adaptation. Permission to perform this translation has been obtained from the original author of the BES.

The Eating Disorder Examination Questionnaire (EDE-Q)

The EDE-Q is a 28-item self-report questionnaire that will be used to evaluate specific ED behaviors and the severity of restraint eating, eating concern, shape concern, and weight concern (75). We will use this to assess to what extent ED behavior is present in our sample and to what extent this impacts the efficacy of semaglutide.

The Dutch Eating Behavior Questionnaire (DEBQ)

The DEBQ is a 33-item self-report questionnaire that will be used to measure three eating behaviors, namely emotional, external, and restraint eating (76). It is the most common questionnaire used to assess eating behavior, and it is different from the EDEQ in the sense that it is not limited to investigating disordered eating behavior.

The Salzburg Emotional Eating Scale (SEES)

The SEES is a 20-item self-report questionnaire that will be employed to assess changes in food intake in relation to discrete emotional states (77). Moreover, we will use this to measure tendencies to increase or decrease food intake during positive and negative emotions, and how these tendencies are influenced by semaglutide.

The Power of Food Scale (PFS)

The PFS is a 21-item self-report instrument that will be used measure food motivation and craving to consume palatable food (78). We will use this to assess how motivated participants are to consume palatable food and how this is affected by semaglutide.

The General Food Craving Questionnaire (trait and state)(G-FCQ-T & G-FCQ-S)

The 39-item G-FCQ-T will be used to measure food cravings as stable traits and the 15-item G-FCQ-S to measure if food cravings are state-dependent (79). We will use these questionnaires to assess if semaglutide affects food craving.

The Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) Questionnaire

The BIS/BAS questionnaire is a 20-item self-report questionnaire that will be used to measure behavioural inhibition or sensitivity to punishment and behavioural approach or sensitivity to reward (80).

The Leeds Food Preference Questionnaire (LFPQ)

The LFPQ will be used to measure food preference. Explicit 'liking' and 'wanting' of food items is determined by presenting VAS with the questions: 'How pleasant would it be to taste some of this food now' and 'How much do you want some of this food now' (81). In addition, we will ask the participants at the end to indicate which of the presented food items they would most like to eat right now.

Questionnaire to assess dietary habit

The Food Frequency Questionnaire (FFQ)

The FFQ will be used to determine the type of food eaten over a specific time and at what frequency (82). We will use this data to analyze whether semaglutide changes what people choose to eat and how often they eat specific food items (such as high-fat or high-calorie food). The other questionnaires mentioned above assess the psychological aspect of eating behavior, whereas the FFQ gives us information about how this is translated into real-life eating behavior.

The Self-Report Habit Index (SRHI)

The SRHI is a 12-item questionnaire developed to assess the strength of habits based on key characteristics such as repetition, automaticity and their connection to personal identity (83). It can be used to measure or monitor habit strength without directly assessing behavior frequency.

Questionnaire to assess emotional state

The Positive and Negative Affect Schedule (PANAS)

The PANAS consists of two 10-item self-report mood scales and will be used to measure state positive affect (PA) and state negative affect (NA) (84).

4.2.4.9 Ambulatory assessments

Experience Sampling Methodology (ESM)

Random and repeated assessments in the natural state and environment of a subject characterize ESM. Unlike previously stated questionnaire-based assessments, ESM has the potential to capture fluctuations in eating behavior and emotions in daily life. We will use the m-Path app (https://m-path.io/landing/research/) to assess the momentary self-report measures during daily life. We will use a m-Path standard premium research account to develop the ESM protocol for our project.

Participants will complete 3 bursts of data collection (T0, T3 and T6), each lasting 3 weeks. During each burst, data is only collected on Thursday, Friday, and Saturday to limit the burden. Per day, participants will receive 8 push notifications ('beeps') from the app sent on a semi-random basis. At each signal, participants will be asked to fill in questions on the following feeling/thought/behavior: negative affect, positive affect, stress, craving, rash action, hunger/satiety, food intake, and BE episodes. These will be scheduled within one of eight 135 min blocks between 8 AM and 11 PM. Participants will be given 15 minutes to respond to the questionnaire, otherwise, it will expire or be overruled by the next notification. Therefore, subjects are instructed to complete as many questionnaires as possible each day, as soon as possible after each beep, but to pass over questionnaires when completion is impossible, e.g. when they are driving. The interval between two consecutive notifications will vary randomly, but between two consecutive blocks, there will be a window of at least 25 minutes. The questionnaires will be identical at each beep, but the order of the questions is randomized. A member of the research team will get in contact with the participant after one day of the ambulatory period to guarantee that everything is running smoothly and there are no technical problems. See Appendix 6 for further information on the ESM procedure and items. Small additional changes might be made after piloting these questionnaires.

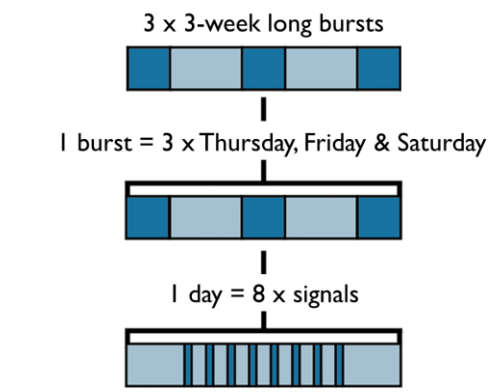


Figure 5. Overview of ESM procedure

Assessment of psychophysiology

Psychophysiological data will be measured using a Garmin wearable: the Garmin vivo active 5 (https://www.garmin.com/nl-BE/p/1057989/pn/010-02862-11/#specs). We will use this wearable to continuously measure ANS activity, more specifically HR and HRV. These measures will be captured during the ESM bursts to pair the momentary self-report data with concurrent psychophysiological measures.

4.2.4.10 Additional assessments

Routine measurements

Routine care data, including anthropometric measurements such as height, weight, waist/hip ratio, body composition (bio-impedance), blood pressure, heart rate, NYHA heart failure classification, medical history including disease and BE duration and medication use will be transferred from the electronic patient file to the pseudonymized study database in REDCap. Routine blood parameters (including fasting glucose, c-peptide, lipids, creatinine/eGFR) will be collected to determine metabolic profile. These basic phenotyping parameters will be used as covariates in analysis (for objectives 1-3) and as inputs in the prediction model for response to therapy (for objective 4).

Routine clinical care

Participants enrolled in the study will continue to receive multidisciplinary obesity care throughout study participation within the Obesity Clinic of UZ Leuven. As per routine clinical care at the Obesity Clinic, participants will be followed by physicians specialized in obesity, dieticians, psychologists and physical therapists. An individualized treatment plan will be presented, taking into account the participants' comorbidities, preferences and physical condition. The Obesity Clinic combines pharmacological obesity treatment with individualized lifestyle, nutritional, physical activity and psychological support. Participants will receive guidance on a healthy diet, eating behaviour and regular physical activity in line with current obesity management recommendations. Psychotherapeutic follow-up may be provided when clinically indicated according to routine clinical practice.

Routine physical activity evaluation

No specific physical activity intervention will be planned as part of this Trial. General evaluation of fitness and physical activity will be based on self-reported minutes of moderate intensity physical activity (with approximately 150 minutes per week recommended if physically feasible) and data from wearable devices (including step count). The study aims to be as inclusive as possible to reflect the real-world patient population entering the obesity clinic. Participants with limitations in physical activity due to disability will not automatically be excluded. However, severe disability or concomitant disease may, on an individual basis, be considered incompatible with specific study procedures (e.g. PET imaging) or overall participation, at the investigator's discretion.

Routine dietary evaluation

Dietary habits will be assessed using a semi-structured interview by the specialized dieticians of the obesity clinic and 7-day recall diary. Such information is routinely recorded in the clinic.

Dietary evaluation

On the three days before an MRI-MMTT visit, participants will be asked to fill in a digital food diary using the Nutritics app. They will also have to do this on the two days before, the day of and the two days after the PET visits. In this food diary, participants will have to enter each food item they eat throughout the day as well as the quantity of each item.

Urine drug test

On each study visit, participants are required to take a urine test to screen for drug use.

Urine pregnancy test

At the screening visit and once per month throughout the Trial, WOCBP are required to undergo a urine pregnancy test to rule out the possibility of pregnancy, as the effects of semaglutide on a developing fetus are not known. At the screening visit, WOCBP will be provided with urine pregnancy tests for monthly remote testing. Participants will receive a monthly reminder by email to perform the test, followed by a link to register their pregnancy test results in REDCap.

Pregnancy is an exclusion criterion and participants with a positive pregnancy test cannot continue in the Trial. If a pregnancy test is positive, participants must immediately contact the study team. On the day of PET scanning, WOCBP are required to undergo a urine pregnancy test to rule out the possibility of pregnancy, ensuring safety and preventing any fetal exposure to radiotracers.

4.3 Premature discontinuation of Trial treatment

Participants may voluntarily discontinue from Trial treatment and/or prematurely end their participation in the Trial for any reason at any time. In such case the Investigator must make a reasonable effort to contact the participant (e.g. via telephone, e-mail) in order to document the primary reason for this decision.

The Investigator may also decide at any time during the course of the Trial, to temporarily interrupt or permanently discontinue the Trial treatment if it is deemed that continuation would be detrimental to, or not in the best interest of the participant.

Circumstances requiring premature treatment interruption or discontinuation of the Trial, include but are not limited to:

- Safety concerns related to IMP or unacceptable intolerability (including BMI < 18.5 kg/m^2)
- Trial participation while in violation of the inclusion and/or exclusion criteria
- Pregnancy
- Intention of becoming pregnant during the Trial and within 2 months after the last semaglutide dose
- Missing 4 consecutive doses of the IMP

In any such case of early Trial termination and/or treatment interruption/discontinuation, the Investigator will continue to closely monitor the participant's condition and ensure adequate medical care and follow-up. It is recommended that follow-up information will be collected as follows:

All AEs will be followed until they have abated or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

If the Trial is prematurely stopped due to an unplanned pregnancy or intention of becoming pregnant, the study medication will immediately be stopped. In case of an unplanned pregnancy after exposure to semaglutide, the participant will again be informed that semaglutide is not approved during pregnancy and that animal studies have shown reproductive toxicity, while available human pregnancy data are limited. In case of an unplanned pregnancy after exposure to semaglutide, counselling will be performed by the high risk obstetric unit (in line with normal routine) and in case of an ongoing pregnancy, an expert ultrasound will be performed at 12 and 20 weeks to screen for congentital anomalies.

In case of an ongoing pregnancy, relevant information on the pregnancy and pregnancy outcomes will be collected in the eCRF (if pregnancy ICF has been provided). In case of a safety issue, the EC will be notified. If a WOCBP discontinues the IMP, highly effective contraceptive methods (see 3.1 inclusion criteria) should be used for 2 months after treatment discontinuation.

For participants whose status is unclear because they fail to appear for Trial visits without stating an intention to discontinue or withdraw, the Investigator must make every effort to demonstrate "due diligence" by documenting in the source documents which steps have been taken to contact the participant to clarify their willingness and ability to continue their participation in the Trial (e.g. dates of telephone calls, etc.).

A participant should not be considered lost to follow-up until due diligence has been completed.

5 Trial Medication / Drug

Generic Drug Name (& company brand name)	**IMP or non-IMP**	**Used within Indication?** (Y or N)	**Route of administration (po,sc,iv,…)**	**Dose/dosage and units**
Semaglutide 3,2 mg/ml (Wegovy®) Novo Nordisk	IMP	Yes	sc	Starting dose is 0.25 mg once a week, escalated every four weeks until the maximal dose of 2.4 mg per week or the highest tolerated dose is reached, which will be maintained until the end of the Trial.

5.1 Investigational Medicinal Product and Dosing Regimen

Semaglutide 3,2 mg/ml (Wegovy®) is a long-acting GLP-1 RA developed by Novo Nordisk for chronic weight management in individuals with obesity or overweight-related comorbidities (85). Semaglutide has been authorized for weight loss by EMA since January 6th 2022. Semaglutide is a modified analogue of human GLP-1, designed for prolonged activity and increased enzymatic stability. Its structure is composed of 31 amino-acids and it retains 94% sequence homology with the native GLP-1 hormone, with three key modifications that enhance its pharmacokinetics:

- Ala8Aib Substitution: Substitution of alanine with 2-aminoisobutyric acid (Aib) at position 8 increases resistance to DPP-4 mediated degradation.
- C18 Fatty Acid Conjugation: A C18 fatty-diacid chain is attached to Lys26 via a γGlu-2xOEG hydrophilic linker. This derivatization promotes albumin binding and reduces renal clearance, ultimately extending the half-life to approximately 7 days.
- Lys34Arg Substitution: Lysine at position 34 is replaced with arginine to optimize production via recombinant technology and prevent C18 fatty-diacid chain binding to the wrong site.

With an extended half-life of approximately 7 days, semaglutide allows for once weekly subcutaneous dosing, improving patient adherence compared to earlier GLP-1 RAs requiring more frequent administration. Wegovy® is administered as a subcutaneous injection containing the active ingredient semaglutide, together with excipients: disodium phosphate dihydrate (E339), sodium chloride, sodium hydroxide (E524), water for injection and hydrochloric acid (E507)(86).

Subjects will be instructed to self-inject the Trial product subcutaneously once weekly in the abdomen, thigh or upper arm. The injection site can be changed without dose adjustment. The subjects must be trained in handling the pen injector when dispensed for the first time and thereafter as indicated during the Trial. Only needles provided by Novo Nordisk must be used for the administration of the Trial product. These needles are provided together with the medication.

Semaglutide treatment will be initiated with a starting dose of 0.25 mg once a week, escalated every four weeks until the maximal dose of 2.4 mg per week or the highest tolerated dose is reached, which will be maintained until the end of the Trial.

If treatment with the Trial product is associated with unacceptable AEs, extensions of dose-escalation intervals, dose reductions and treatment pauses are allowed at the discretion of the principal investigator.

The investigator should consider delaying the dose escalation or reducing the dose if the current dose is not tolerated. During dose escalation and during dose maintenance, efforts should be made by the investigator to support participants in escalating and maintaining the corresponding IMP dose.

If GI AEs occur during dose escalation, dose escalation may be postponed and participants may remain on the previously tolerated dose according to the investigator's judgement and treatment tolerability. To mitigate and manage GI symptoms, participants can be advised to eat more slowly, eat smaller meals and stop eating when feeling full. Symptomatic treatment (e.g., antiemetic or antidiarrhoeal medication) may be initiated if clinically indicated. In case of clinically relevant or intolerable GI AEs, temporary interruption or discontinuation of semaglutide may be considered by the investigator.

In the event of a medical procedure or temporary interruption of the treatment, it will be discussed with the principal investigator whether re-initiation is possible after recovery.

If a dose is missed, it should be administered as soon as possible and within 5 days after the missed dose. If more than 5 days have passed, the missed dose should be skipped and the next dose should be administered. Missing more than 3 consecutive doses will be recorded as a protocol deviation. If a participant misses 4 consecutive doses, they will be withdrawn from the Trial. Recommended actions for missed consecutive doses are outlined below.

Missed dose situation	Recommended action		Study implication
	Time since last dose	What dose to administer	
Participant misses a single dose or 2 consecutive doses of IMP	If time since last dose is <3 weeks (21 days)	Administer the same dose of IMP	N/A
Participant misses 3 consecutive doses of IMP	If time since last dose is ≥3 weeks (21 days) but <4 weeks (28 days)	Restart IMP one dose step lower than the last dose administered	Protocol deviation
Participant misses 4 or more consecutive doses of IMP	If time since last dose is ≥4 weeks (28 days)		Trial discontinuation

The injection can be administered at any time of the day, irrespective of meals, but on the same day of the week. The day of weekly administration can be changed if necessary as long as the time between two doses is at least 2 days (>48 hours). After selecting a new dosing day, once weekly dosing should be continued.

The FlexTouch prefilled pen available in 1.5 mL (0.25 and 0.5 mg) and 3 mL (0.5, 1, 1.7 and 2.4 mg) formats will be used. These pens will be purchased directly by the study team and will not be provided free of charge by Novo Nordisk. Each pen contains a type I glass cartridge, sealed at one end with a chlorobutyl rubber plunger and at the other end with an aluminum cap incorporating a laminated rubber layer of bromobutyl/polyisoprene. The cartridges are mounted in a prefilled disposable pen made from polypropylene, polyoxymethylene, polycarbonate and acrylonitrile-butadiene-styrene.

For all participants, semaglutide training, dispensing of the 6-month supply of semaglutide pens and the first semaglutide injection will all take place at the end of their baseline MRI–MMTT visit. Further, for all participants, the 6-month semaglutide MRI–MMTT visit must take place while the participant is still on active treatment. Therefore, this visit should be scheduled within 6 days after the participant's last semaglutide injection. If scheduling this visit within this 6-day window is not feasible due to practical constraints, such as scanner availability, the participant may receive an additional semaglutide dose in order to maintain active treatment until the visit can be conducted.

5.2 Drug Accountability

Only subjects enrolled in the Trial may receive the IMP and only authorised site staff may supply the IMP. The principal investigator must confirm that appropriate temperature conditions have been maintained during transit for all Trial products received and any discrepancies are reported and resolved before use of the IMP.

All Trial products must be stored in a secure, controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the principal investigator and authorised site staff in line with the storage conditions on the label. If the temperature excursion is outside of the accepted temperature interval, a temperature deviation must be reported.

Subjects must return all used and unused Trial products as instructed by the investigator. Moreover, used pen injectors must be placed in the provided needle containers and returned in the container, whereas unused pen injectors must be returned separately. The investigator is responsible for drug accountability and record maintenance (i.e. receipt, accountability and final disposition records). Drug accountability must be performed in the eCRF by registering pen-injectors as returned either as used or unused, or as lost.

Destruction of IMPs can be performed on an ongoing basis and will be done according to local procedures after accountability is finalised. Destruction of IMPs must be documented in the eCRF and drug accountability log.

All returned, expired or damaged IMPs must be stored separately from non-allocated Trial products. No temperature monitoring is required. Non-allocated IMPs, including those that are expired or damaged, must be accounted as unused.

5.3 Concomitant / Prohibited Medication / Treatment

For all prohibited medication see Section 3.2. During the study, participants should not initiate any anti-obesity treatment that is not the IMP. If such treatment is initiated, the participant will be instructed to stop the anti-obesity treatment immediately.

5.4 Rescue Medication

Not applicable

6 Safety reporting

6.1 Definitions

6.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or subject during a Trial, and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a product, whether or not considered related to the product. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE.

6.1.2 Adverse Reaction (AR) or Adverse Drug Reaction (ADR)

An AR is any noxious and unintended responses to an investigational medicinal product or to a Trial and, when an investigational product is concerned, related to any dose administered.

6.1.3 Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that results in any of the following:

- Inpatient hospitalisation or prolongation of existing hospitalization
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- A life-threatening[a] experience
- Death
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they may jeopardise the subject and may require medical or surgical intervention to prevent one of the above outcomes

[a] *The term "life threatening" in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.*

6.1.4 Serious Adverse Reaction (SAR)

A SAR is an Adverse Reaction that meets the criteria for a Serious Adverse Event (as defined under SAE).

6.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is a serious adverse reaction, the nature, severity or outcome of which is not consistent with the applicable product reference safety information (e.g. investigator's brochure (IB) for an unauthorised

investigational product or the patient leaflet joined to the summary of product characteristics (SmPC) for an authorised product).

Therefore, a SUSAR is assessed by the sponsor and/or investigator as:

- **Serious**: The event is classified as an SAE.
- **Unexpected**: The nature, severity or outcome of the adverse event is not consistent with the information provided in the IB or SmPC.
- **Suspected (causality)**: There is a reasonable possibility that the adverse event may be related to the IMP.

6.1.6 Adverse Events of Special Interest (AESI)

The following events should be reported within the same timelines as SAEs:

- Overdose
- Misuse/abuse
- Pregnancy

6.1.7 Unexpected events that might influence the benefit-risk balance

The following unexpected events that might materially influence the benefit-risk balance, and which are not considered SUSARs, should be reported to the Member States:

- Increase in rate of occurrence of expected serious adverse events which may be clinically important
- Significant hazard to patient population

Unexpected events that are likely to seriously affect the benefit-risk balance and emergency safety measures which have been taken to protect the subjects are considered urgent safety measures.

6.2 Adverse Events that do not require reporting

In general, the following should not be reported as AEs:

- Pre-existing conditions, including those found as a result of screening (these should be reported as medical history or concomitant illness). However, in case the pre-existing condition appears to be enhanced by the IMP, this should be reported as an AE.
- Pre-planned procedures, unless the condition for which the procedure was planned has worsened from the first Trial-related activity after the subject has signed the informed consent.

The following events are commonly observed and are therefore not considered as adverse events for the purpose of the Trial:

- All events or reactions listed in the Summary of Product Characteristics (SmPC) of Semaglutide 3,2 mg/ml (Wegovy®)

Although these events should not be reported to the Sponsor, these should be recorded in the patient's medical notes according to routine practice.

The following events not to be considered as SAEs are:

- Pre-planned hospitalisations unless the condition for which the hospitalisation was planned has worsened from the first Trial-related activity after the subject has signed the informed consent.
- Hospitalisation as part of a standard procedure for protocol therapy administration. However, hospitalisation or prolonged hospitalisation for a complication of therapy administration will be reported as an SAE.
- Hospitalisation or prolongation of hospitalisation for technical, practical, or social reasons, in absence of an AE.

6.3 Recording and reporting of Adverse Events

Investigators will seek information on AEs during each patient contact. All events, whether reported by the patient or noted by Trial staff, will be recorded in the patient's medical record and in the (e)CRF within a reasonable time after becoming aware. The diagnosis rather than the individual signs or symptoms should be reported, if available. If no diagnosis is available, the Investigator should record each sign and symptom as individual AEs.

The following minimum information should be recorded for each AE:

- AE description
- start and stop date of the AE
- severity
- seriousness
- causality assessment to the Investigational Medicinal Product (IMP) and/or Trial procedures
- outcome

6.3.1 Assessment

All AEs must be evaluated by an Investigator as to:

- **Seriousness:** whether the AE is an SAE. See above for the seriousness criteria.

- **Severity:** Severity must be evaluated by an Investigator according to the following definitions:
  - *Mild* – no or transient symptoms, no interference with the subject's daily activities
  - *Moderate* – marked symptoms, moderate interference with the subject's daily activities
  - *Severe* – considerable interference with the subject's daily activities, unacceptable
- **Causality:**
  - *None* – An AE which is not related to the IMP or the Trial
  - *Unlikely* – An AE for which an alternative explanation is more likely (e.g. concomitant medication(s), concomitant disease(s)), and/or the relationship in time suggests that a causal relationship is unlikely
  - *Possible* – An AE which might be due to the use of the IMP or Trial participation. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be ruled out.
  - *Probable* - An AE which might be due to the use of the IMP or Trial participation. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely.
  - *Definitely* – An AE which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation. The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge).

6.3.2 Timelines for reporting

- After informed consent has been obtained but prior to initiation of Trial treatment, only adverse events caused by a Trial specific procedure should be reported.
- After initiation of Trial treatment, adverse events will be reported as follows:
  - From start of IMP treatment until visit 4/5 at 6 months: all AEs, SAEs and AESIs will be collected.
  - At 3 months, a telephone follow-up will be conducted to assess and record AEs, SAEs and AESIs.

All SAEs and AESI as defined in the protocol must be reported to the Sponsor within 24 hours of the Trial staff becoming aware of the event. The immediate report shall be followed by detailed, written reports. The immediate and follow-up reports shall identify subjects by Trial identification.

SAE details will be reported by the Investigator to the Sponsor:

- By completing the SAE form in the (e)CRF

If an authorised Investigator from the reporting site is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor by a healthcare professional within 24 hours of becoming aware of the SAE, but must be followed-up by medical assessment as soon as possible thereafter.

Unexpected events affecting the benefit-risk balance must be reported ad hoc to the sponsor as soon as possible after becoming aware of the event.

6.3.3 Follow-up

The Investigator must record follow-up information by updating the patient's medical records and the appropriate forms in the (e)CRF. The worst case severity and seriousness of an event must be kept throughout the Trial.

SAE follow-up information should only include new (e.g. corrections or additional) information and must be reported within 24 hours of the Investigator's first knowledge of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- All *SAEs* must be followed up until the outcome of the event is 'recovered', 'recovered with sequelae', 'not recovered' (in case of death due to another cause) or 'death' (due to the SAE) and until all related queries have been resolved, or until end of Trial (whichever occurs first).
- *Non-serious AEs* must be followed up until the patient's last Trial visit, and until all related queries have been resolved.

SAEs after the end of the Trial: If the Investigator becomes aware of an SAE with suspected causal relationship to the IMP or the Trial after the subject has ended the Trial, the Investigator should report this SAE within the same timelines as for SAEs during the Trial.

6.3.4 Pregnancy

Female subjects must be instructed to notify the Investigator immediately if they become pregnant during the Trial. The Investigator must report any pregnancy in subjects who have received Trial product(s) to the Sponsor within 24 hours of becoming aware of the event.

6.3.5 Technical Complaints

Technical complaints can be reported to the Marketing Authorisation Holder (eg. change in color, unequal sizes, broken vials/pills, sedimentation, …).

6.3.6 Death

All deaths will be reported without delay to the sponsor (irrespective of whether the death is related to disease progression, the IMP, Trial procedure or is an unrelated event).

6.4 Reporting requirements to the Member states

The Investigator is responsible for ensuring that all safety events are recorded in the (e)CRF and reported to the Sponsor in accordance with instructions provided below.

The Sponsor will promptly evaluate all SAEs and AESIs against medical experience to identify and expeditiously communicate possible new safety findings to the Investigators.

6.4.1 Sponsor's reporting of Suspected Unexpected Serious Adverse Reactions (=SUSARs)

After receiving the SAE report form with causality (relationship) assessment from the Investigator, the sponsor can also make a causality assessment. The term SADR (Serious Adverse Drug Reaction) is to be

used whenever either the Investigator or the Sponsor deems the SAE as possibly or probably related to the IMP.

The Sponsor must evaluate (and document the evaluation of) the expectedness for each SADR against the Reference Safety Information, e.g. in the Investigator's Brochure or applicable product information. In case the event is Unexpected (= a SUSAR) it must be reported by the Sponsor to Member states (through the EudraVigilance database) and other participating Investigators within the following timelines:

- **7** calendar days if fatal or life-threatening event (follow-up information within an additional 8 days)
- **15** calendar days if non-fatal or non-life-threatening event (follow-up information as soon as possible)

For reporting to the EudraVigilance database, all information related to the SUSAR should be provided by the Sponsor to the CTC of UZ Leuven as soon as possible. Contact details: CTC.safety@uzleuven.be and tel. 016 34 19 98.

6.4.2 Sponsor's reporting of unexpected events that might influence the benefit-risk balance

The sponsor has the obligation to notify the Member states through the EU portal (CTIS) for all unexpected events, that are not SUSARs, and which affect the benefit-risk balance within the following timelines:

- **7** calendar days for serious urgent safety measures
- **15** calendar days for other events

6.4.3 Temporary halt or early termination of the clinical Trial for reasons of subject safety

The sponsor has the obligation to notify the concerned Member States through the EU portal (CTIS) in case of early termination of the clinical Trial for safety reasons. The notification (containing information on the reasons for such action and follow-up measures) should be made immediately, but not later than 15 calendar days after the temporary halt or termination.

6.4.4 Annual reporting

The Sponsor has the obligation to, once a year throughout the clinical Trial (or on request), submit a Development Safety Update Report (DSUR) through the EU portal (CTIS), taking into account all new available safety information received during the reporting period.

6.4.5 Overview reporting requirements

	WHAT	HOW	TO	TIMELINES
Investigator	AE	AE form	sponsor	asap, but not later than 7 calendars days
	SAE	SAE form	sponsor	Immediately (within 24 hours of becoming aware of the event) Exceptions: as defined in the protocol
	death	SAE form	sponsor	asap
	Unexpected events affecting the benefit-risk balance	ad hoc	sponsor	asap

Sponsor	SUSAR	EudraVigilance	**unblinded:** - Member states (via EudraVigilance)	asap, but no later than - 7 calendar days (in case of fatal or life-threatening SUSAR) - 15 calendar days (other SUSAR)
	Unexpected events affecting the benefit-risk balance	EU portal (CTIS)	EU portal (CTIS)	asap, but not later than - 7 calendar days (serious urgent safety measures) - 15 calendar days (other unexpected events)
	Annual Safety/ Development Safety Update Report	DSUR template	EU portal (CTIS)	annually

6.5 Data Safety Monitoring Board (DSMB)

After a thorough evaluation of the potential risks to Trial participants and considering the nature, design and complexity of the clinical Trial, the Sponsor has determined not to establish a DSMB. This decision is grounded in our conclusion that this Trial is low risk for participants, as it involves the use of semaglutide, a drug with a well-characterized safety profile. Semaglutide, the investigational GLP-1 RA, is widely used in Belgium and internationally for the treatment of obesity in normal routine with well-known side-effects. All potential side-effects will be closely monitored throughout the study to ensure timely detection and management.

Imaging procedures are low risk: PET scans involve minimal radiation exposure (maximum 4.725 mSv, WHO IIb classification), and participants with excessive prior exposure will be excluded. MRI scans do not involve ionizing radiation, and strict screening for contraindications (e.g., metallic implants, claustrophobia) will be applied. Incidental findings will be communicated to participants' treating physicians.

Blood sampling via intravenous or arterial catheter is a routine medical procedure. Complications are typically mild (bruising, minor bleeding, swelling), with vasovagal reactions mitigated by performing procedures in a reclined position. Wearables, hair and urine sample collection, and questionnaires carry minimal or negligible risk. Mild skin irritation from wristbands is managed by advising temporary removal. Completing ESM diaries may be burdensome but is non-invasive, and participants are instructed to pause if unsafe to respond.

All procedures will follow GCP, radiation protection guidelines, and institutional protocols. Given the well-known safety profile of semaglutide, low-risk procedures and active monitoring measures, a DSMB is not expected to add meaningful value to participant safety or data integrity for this study.

The Trial is committed to ensuring the safety and well-being of all participants at all times by adhering to the latest version of GCP and other relevant and applicable standards and legislations.

7 Statistics and Data Analysis

Statistical analysis will be performed in accordance with ICH E9; a detailed description of the analysis is provided in the Trial-specific Statistical Analysis Plan (SAP). ICH E3 and E8 will guide the structure and content of the clinical Trial report.

7.1 Sample Size Determination

A total of 129 patients will be included in the Trial to account for an expected 25% dropout, ensuring that 96 evaluable patients complete the Trial.

A priori sample size calculations require reliable estimates of the expected effect size from previous studies, which are sparse. Moreover, effect sizes on the effect of lira- or semaglutide on BE and other eating behaviors vary widely between studies (Cohen's d = 0.31-1.55). In the only study on patients across the BE spectrum, a large effect size (d=0.94) was found (87). A sensitivity power analysis in GPower 3.1.958 indicates that our sample size (n=96 after accounting for dropout, we will overrecruit 25%) yields 91% power to detect the smallest abovementioned effect size (d=0.31) on BES (Aim 1.2.) in a paired t-test (baseline vs at 6 months of treatment) (88). The effect sizes for semaglutide on anthropometric outcomes are (very) large (21).

A priori effect sizes for the hypothesized mediating effect of psychobiological processes underlying eating behavior are lacking or impossible to calculate from the sparse literature, both for path a (semaglutide → psychobiological process) and path b (psychobiological process → anthropometric and BE outcome) of the mediation model. However, Monte Carlo simulation-based power calculations for within subject mediation analyses show that our proposed sample size yields >80% power for medium effects (standardized β = 0.30) for both paths, using the power calculation method provided by using the SAS macro MEMORE. For brain mediators (PET, fMRI), we are not aware of a power calculation method, but our sample sizes for the PET and fMRI studies are the largest of their kind. Further, we will use the powerful multivariate mediation method "principal directions of mediation", as successfully applied to data of similar or lower sample sizes by our lab (89–91).

A similar lack of effect sizes applies to the moderation analyses (Aim 1.3.). A sensitivity power analysis for within subject moderation in GPower indicates that our sample size yields 80% power to detect small to medium effects (standardized β = 0.27; Cohen's $f^2 < 0.08$).

We are not aware of a power calculation method for the machine learning algorithms we will use to develop the prediction model. However, a sensitivity power analysis in GPower for a simpler multiple regression model predicting the continuous outcomes indicates that our sample size yields 80% power to detect medium effects (Cohen's $f^2 = 0.23$) for 15 predictors. This underestimates the power of the machine learning models, as they handle the likely correlated nature of the predictors better.

Generally, for several mediators and outcomes, such as hormone and ESM data, many repeated measurements will be available, allowing for the use of powerful linear mixed models and structural equation models, further increasing statistical power. Moreover, continuous predictor and outcome variables will be used consistently, as they do not require arbitrary cutoffs and maximize power by retaining all true variability in the data.

7.2 Statistical Analysis

Statistical analyses will be done in Statistical Analysis System (SAS), R, JMP, JASP, and in Matlab/Statistical Parametric Mapping (SPM) for brain imaging data. For the latter, we will use toolboxes developed by the Cognitive and Affective Neurosciences lab ([redacted]) and the LaBGAS. PET data will be analyzed using the linearized simplified reference region model (LSRRM) in Matlab. ESM data will be analyzed using linear mixed models and structural equation models using R scripts.

Objective 1

To assess Aim 1.1., between-subject variability (e.g., standard deviation) will be computed for all outcome measures, and within-subject variability will be examined for the ESM data to capture individual fluctuations over time. Aim 1.2. will be addressed using linear mixed models with time (T0, T3, T6) as a within-subject independent variable and anthropometric measures and BE severity (change from baseline) as dependent variables, while controlling for baseline values. The impact of additional basic phenotyping parameters will be explored by including them as covariates in the models. For Aim 1.3., moderation analyses will be conducted by adding baseline eating behavior measures and their interaction with time to the models described in Aim 1.2., with anthropometric measures as dependent variables. A significant interaction term will indicate a moderating effect.

Objective 2

For Aims 2.1. to 2.10., mediation analyses will be conducted with time (T6 versus T0, reflecting the semaglutide effect) as the independent variable (X), anthropometric measures and BE severity as dependent variables (Y), and eating behavior measures along with their underlying motivational psychobiological processes as mediators (M). If a mediator consists of repeatedly measured within-subject data, participant-level summary measures will be calculated or multilevel mediation analysis will be applied as implemented in the CANlab Mediation Toolbox. Mediation analyses will be performed using the SAS macro MEMORE and the CANlab Mediation Toolbox for brain imaging data. For brain imaging mediators, a multivariate method called "principal directions of mediation" will be used to decompose brain signals into components that maximally mediate the X-Y relationship, as already implemented in our research group (90). If multiple mediators are identified, serial, parallel, or moderated mediation models will be applied using the SAS macro MEMORE to explore the relationships between the different mediators.

Objective 3

For Aim 3.1., moderated mediation analysis will be conducted to test whether the mediation effects identified in Aims 2.1. to 2.10., specifically the pathway from semaglutide effect (X) through psychobiological processes (M) to anthropometric outcomes (Y), depend on the level of the moderator baseline BE severity (92). These conditional indirect effects will be implemented using the SAS macro MEMORE.

Objective 4

To address Aim 4.1, predictive regression machine learning algorithms will be trained using k-fold cross-validation (including random forest, gradient boosting, neural networks, partial least squares, and Least Absolute Shrinkage and Selection Operator (LASSO) principal components regression (PCR)) on features extracted from the basic phenotyping parameters, and baseline measurements of eating behavior and its underlying three motivational psychobiological processes. Performance of the models will be compared based on explained variance in the dependent variable and the relative contribution of the different features (variable worth) will be quantified. Experience with implementing such models is available in our research groups, both on behavioral data (using SAS Enterprise Miner and JMP (add-ons)) and brain imaging data (using Matlab-based CANlab tools), as well as on ESM data using R (93–95).

7.2.1 Efficacy Analysis

Endpoint	Statistical Analysis Methods
Primary	(Generalized) linear mixed models
Secondary	Mediation, moderation, and moderated mediation analyses
Exploratory	Not applicable

7.2.2 Other Analysis

Not applicable

7.3 Interim Analysis and Final Database Lock (if applicable)

Not applicable

8 Data handling

Data handling and data flows for the Trial are summarized below and will be described in more detail in the Trial-specific Data Management Plan (DMP).

All data will be treated confidentially and pseudonymised (i.e. coded) using the Research Electronic Data Capture (REDCap) software. After passing screening and providing informed consent, all participants will be assigned a unique individual numeric code to ensure the anonymity of the subjects during the analysis of the samples and data. This record, as well as most data files, will be stored in the REDCap database. Access will be restricted to project members, who will be able to link these data to participants' direct identification information (e.g. names or contact details) for the purpose of the study and for their reimbursement.

In the event of technical failure, data collection may revert to paper-based methods (e.g. self-report questionnaires). Again, those documents will only mention numeric participant codes, not individual names, allowing the linking of such data and experimental results without access to participants' identities. Paper documents will be stored in key-locked cabinets on university premises, in rooms that are not publicly accessible (office rooms). It will not be possible for anyone to discern which participant produced which data from the data files without access to the separate record that links individual codes to personal identification data. These documents will be computerized into REDCap.

All data that is collected digitally (e.g. informed consent form, questionnaires and in- and exclusion criteria) will be collected using REDCap. REDCap is a secure web application preferred by KU Leuven and UZ Leuven for building and managing online surveys and databases.

All biological samples (i.e. blood, urine and hair) are coded as described above, and blood and urine samples are temporarily stored in freezers at KU Leuven.

Raw imaging data files will be briefly stored on individual, password-protected computers used for data acquisition (during testing). Afterwards, they will be transferred to the dedicated secure drive. Data on those servers are automatically backed up by the central university ICTS services. Data may also be posted to public servers upon publication of research results, as per request of an increasing number of scientific journals, to ensure reproducibility and scientific integrity. In addition, all analysis scripts will be made publicly available on our lab's Github page (https://github.com/labgas) in such publications. In such cases, again, it will not be possible to link any data to any individual, as all personal identification data will be removed.

M-Path (https://m-path.io/landing/) is developed at KU Leuven and consists of (1) a secure mobile platform that clients can use on their phones for filling out assessments in real-time and real-life, and (2) a secured dashboard in which health care professionals can access to visualize the obtained ESM data. All ESM data will be collected with the m-Path app (i.e., questionnaire data). Data is initially stored locally in a protected folder on the smartphone of the participant that can only be accessed through the m-Path app. This folder cannot be accessed through other apps. The daily diary data that are stored on the participant's personal phone are automatically deleted once the app is removed or uninstalled from the phone. Participants will be informed that if they wish to permanently delete their own data, they will have to uninstall the app. As one of the benefits of the study is that participants gain insight into their own emotional and behavioral trajectory, which is visualized in the app, they may want to keep this information for personal use. We, therefore, will not force participants to uninstall the app but instruct them that it is recommended to remove the data from the phone as soon as they do no longer feel the use of it. As the legal owner of their data, participants have the right to decide, at any time, to make the data collected using m-Path unavailable to the researchers. Furthermore, participants have, at any time, the option to delete their data from the m-Path database. These actions can be taken inside the m-Path app, by following a few simple steps. Participants will be explained how to do this if they wish to make use of this right. To enhance data security and to prevent data leakage at all times, a highly secured application-layer encryption is applied. All answers given to questionnaires, all downloaded questionnaires, personal information (i.e. alias), options and notes are stored on the phone using AES 256 bit-encryption with PKCS7 padding. This means that the stored data itself consists of bytes without any meaning. If the user has access to a 3G network or above, data is transferred to secured university servers located in Leuven and Heverlee. In the unlikely event of a security breach, all affected users will be notified.

Psychophysiological data will be collected using the Garmin Vivoactive 5 smartwatch and will automatically synced via Bluetooth at random time points to the application programming interface (API), Fitrockr (https://www.fitrockr.com/). The data will be securely stored on the Fitrockr platform for the duration of the study on servers located in Europe. Fitrockr is ISO and HIPAA certified and follows all requirements for GDPR compliance in the processing and storage of research data. All data on Fitrockr is pseudonymised as it only requires a unique numerical participant code to create a profile. After researchers have set up the app by linking the smartwatch to a Fitrockr profile through a QR code, participants will not need to engage with the app ever again. Further, the Fitrockr platform provides a blinded display mode that only shows the time and the battery level on the smartwatch, which prevents both participants and others from viewing any physiological data. This not only reduces the potential for reactivity but also adds an additional layer of data protection. Only the research team can access the data and neither Garmin nor Fitrockr can use or distribute it in any form. Fitrockr will permanently delete all data following completion of analysis and publication.

Paper documents, electronic files (including processed data and analysis scripts) and biological samples will be retained for minimally 25 years after the conclusion of data collection. They will eventually be destroyed using dedicated university procedures for the destructing of biological samples and sensitive documents (in case of paper documents) or by the erasure of electronic files from the back-up drive.

All research data will be de-identified for analysis. As specified in the informed consent, the participant is required to give permission for the scientific use of the data and is informed that anonymity and confidentiality will be ensured at all times. The pseudonymized data will be securely stored separately from the direct identification data on a shared and encrypted network drive (KU Leuven) that is only accessible to involved researchers. Pseudonymized data will not be transferred unencrypted or by email. Pseudonymized data can also be shared with other parties that collaborate with the research team. Information with regard to data sharing will be part of the informed consent.

8.1 Data Collection Tools and Source Document Identification

8.1.1 Operational aspects

Data collection, handling, processing and transfer for the purpose of this Trial will be performed in compliance with applicable regulations, guidelines for clinical Trials and internal procedures, as follows:

8.1.1.1 Data collection

Source Data will be collected and recorded in the Trial participant's files/medical records.

If applicable, worksheets may be used for capturing some specific data in order to facilitate completion of the (e)CRF. Any such worksheets will become part of the Trial participant's source documentation and will be filed together with or as part of the medical records (during but also following completion of the Trial).

It remains the responsibility of the Investigator to check that all data relating to the Trial, as specified in the Trial protocol, are entered into the (e)CRF in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

REDCap will be used as the (e)CRF. REDCap is a secure, web-based application preferred by KU Leuven and UZ Leuven for building and managing online surveys and databases.

(e)CRFs are provided by the Sponsor for each participant. The Trial data will be transcribed from the source records (i.e. participant's medical file or Trial-specific source data worksheets) into an (e)CRF by Site Trial Staff. Transcription to the (e)CRF will be done as soon as possible after a participant visit and in a pseudonymized manner using a unique identifier assigned by the Sponsor.

8.1.1.2 Data Validation

All data relating to the Trial must be prepared and validated by the Investigator. Any (e)CRF entries, corrections and alterations must be made by the Investigator or other authorized Trial staff.

Proper audit trails must be available to demonstrate the validity of the Trial data collected. This includes historical records of original data entries, by whom and when the data was entered, as well as detailed records of any corrections or additions made to the original data entry (i.e. who made the correction/addition, when and why), without obliterating the original data entry information.

8.1.1.3 Data Management

The Trial Data Manager will perform extensive consistency checks on the received data. Queries will be issued in case of inconsistencies in accordance with internal procedures. A Data Management Plan (DMP) will be developed to map data flows, data validation measures that will be taken, how (interim) database lock(s) will be managed and, as applicable, the role and responsibilities of the Data Safety Monitoring Committee (DSMB).

8.1.1.4 Data Transfer

Any participant records or datasets that are transferred to the Sponsor or any partners of the Sponsor will contain the Trial-specific participant identifier only; participant names or any information which would make the participant identifiable will not be transferred. All pseudonymized data relating to the Trial must

be transmitted in a secure manner to the Sponsor or any partners of the Sponsor(see 8.1.2. legal requirements).

8.1.2 Legal requirements

All source data will be kept at a secured location with restricted access at all times. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data protection laws and regulations and more in particular the GDPR and relevant national laws implementing the GDPR. Appropriate technical and organizational measures to protect the data against unauthorized disclosure or access, accidental or unlawful destruction, or accidental loss or alteration must be established. Trial staff whose responsibilities require access to personal data agree to keep the data confidential.

The Investigator and the Participating Site(s) (as applicable) shall treat all information and data relating to the Trial disclosed to them as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the objectives of the Trial as described in this protocol. The collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with applicable laws and regulations regarding personal data protection and the processing of personal data.

The Investigator will maintain all source documents and completed (e)CRFs that support the data collected from each Trial participant, and will maintain a Trial Master File (TMF)/Investigator Site File (ISF) containing all Trial documents as specified in GCP Chapter 8 entitled "Essential Documents for the Conduct of a Clinical Trial", and as specified by applicable regulatory requirement(s).
The Investigator will take appropriate measures to prevent accidental or premature destruction of these documents.

Transfer of the pseudonymized data will be performed via a secured method of transfer taking into account all applicable security arrangements and regulations (such as the GDPR).

8.2 Audits and Inspections

The Investigator will permit direct access to Trial data and documents for the purpose of monitoring, audits and/or inspections by authorized entities such as but not limited to: the Sponsor or its designees and competent regulatory or health authorities. As such (e)CRFs, source records and other Trial related documentation (e.g. Investigator Site File, the Trial Master File, pharmacy records, etc.) must be kept current, complete and accurate at all times.

8.3 Monitoring

In accordance with GCP the Sponsor is responsible for monitoring the Trial to ensure compliance with GCP and current legislation, and to verify, among other requirements, that proper written informed consent has been obtained and documented, that the Trial procedures have been followed as shown in the approved protocol, and that relevant Trial data have been collected and reported in a manner that assures data integrity. UZ Leuven Clinical Trial Center (CTC) performs a risk analysis to determine the monitoring strategy. Based on this risk assessment, the clinical trial was classified as 'low risk' as the potential risks associated to study procedures and protocol design are comparable to that of standard medical care since dosage and administration of IMP is well-known in this patient population (standard of care, phase 4 trial). Hence, based on the above risk assessment and as permitted by GCP, the Sponsor of the trial accepts the minimal risks associated with this trial and determines that monitoring activities (as defined by GCP by a qualified individual, independent of the study team, is not necessary as it will provide little or no added value in protecting the safety of trial participants and assuring the integrity of collected trial data. Nonetheless, the UZ Leuven study team will take all possible measures to assure the quality and integrity of trial data and to safeguard the safety and wellbeing of trial participants, in accordance with the requirements set out in GCP.

8.4 Archiving

As specified in GCP, the Sponsor and Investigator/Participating Site will maintain a record of the location(s) of all respective Essential Trial Documents (including but not limited to Source Documents, completed and final (e)CRF and ISF/TMF). The Sponsor should ensure that the Investigator has control of and continuous access to the (e)CRF data reported to the Sponsor during the Trial.

The Investigator/Participating Site should have control of all Essential Documents and records generated by the Investigator/Participating Site before, during and following termination of the Trial.

Trial specific documentation (such as but not limited to the Trial protocol, any modifications thereto, the final Clinical Study Report (CSR) and the Trial database) shall be archived. Source data and site-specific Trial documents (such as but not limited to the original signed ICFs) will be archived by the participating site(s) according to local practice, and for at least 25 years following termination of the Trial. Archived data may be held on electronic record, provided that media back-up exists, hard copies can be obtained, if required and measures are taken to prevent accidental or premature loss or destruction of data. Destruction of Essential Documents prior to, during or upon completion of the required archival period, will require written authorisation from the Sponsor.

9 Ethical and Regulatory Considerations

9.1 CA/EC review & reports

Before the start of the Trial, this protocol and other related documents (e.g. ICF, advertisements, IB, etc.) will be submitted through the CTIS portal for review to the CA/EC for Trial authorization. The Trial shall not commence until such approvals have been obtained and until other relevant essential Trial documents, such as duly signed contract agreements, evidence of adequate Trial financing etc. are in place.

It is the responsibility of the CI to produce the Development Safety Update Report (DSUR) and submit to the EC/CA through CTIS within 60 days of the anniversary date on which favourable opinion to start the Trial was given, and annually until the Trial is declared ended.

9.2 Peer review (if applicable)

Not applicable

9.3 Regulatory Compliance

The Trial will be conducted in compliance with the principles outlined in the requirements for the conduct of clinical Trials in the EU as provided for the CTR and any subsequent amendments, as well as in compliance with GCP guidelines, other GxP guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2017 on clinical Trials with medicinal products for human use and with the GDPR, the relevant Belgian laws implementing the GDPR, including the Belgian Law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data and the Belgian Law of August 22nd 2002 on patient rights and all other applicable legal and regulatory requirements.

9.4 Protocol / GCP compliance

The Trial must be performed in accordance with the protocol, current ICH and GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of Trial participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the Trial data are credible, reliable and reproducible.

The Investigator and Trial team acknowledge and agree that prospective, planned deviations or waivers to the protocol are not permitted under applicable regulations on clinical studies. However, should there be an unforeseen protocol deviation, such deviation shall be adequately documented in the source documents and on the relevant forms and reported to the CI and Sponsor during the Trial. Deviations should also be reported to the EC as part of the EC's continued review of the Trial (e.g. through the DSUR). Protocol deviations which are found to frequently recur, will require (immediate) action. The Investigator acknowledges that such recurring protocol deviations could potentially be classified as a major protocol deviation or serious breach. The Investigator and Trial team understand that in case of a serious breach (as defined in the CTR, an event that is likely to affect to a significant degree the safety or rights of Trial participant(s), or the reliability and robustness of the data generated in the clinical Trial), immediate action is required. If serious breaches should occur, this shall be appropriately documented and escalated to the Sponsor as soon as possible. In accordance with Art 52 of the CTR, the sponsor shall notify the participating

Member States using CTIS without undue delay and not later than 7 days after becoming aware of the breach.

The Investigator is expected to take any immediate action required to protect the safety of any participant included in the Trial, even if this action represents a deviation from the protocol. In such cases, the Sponsor should be notified of this action.

The Sponsor has performed a risk assessment to reduce anticipated risks with regards to the safety, wellbeing and rights of the participants as well as with regards to the reliability and robustness of the clinical Trial data.

9.5 Data protection and participant confidentiality

The Trial will be conducted in compliance with the requirements of the GDPR, the relevant Belgian laws implementing the GDPR including the Belgian Law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data. Any collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with the aforementioned personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix). In case personal data is transferred outside the European Economic Area, safeguards will be taken to ensure that appropriate protection travels with the data in accordance with the GDPR. (https://ec.europa.eu/info/law/law-topic/data-protection/international-dimension-data-protection/rules-international-data-transfers_en#documents)

Any personal data shall be treated as confidential at all times including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

9.6 Insurance

The Participating Site, the Investigator and Sponsor shall have and maintain in full force and effect during the term of this Trial, and for a reasonable period following termination of the Trial, adequate insurance coverage for: (i) medical professional and/or medical malpractice liability, and (ii) general liability.

For Belgian Participating Sites

Art 12 of the Belgian Law of May 7th 2017 on clinical Trials with medicinal products for human use applies. Prior to the start of the Trial, the Sponsor shall enter into an insurance contract in order to adequately cover Trial participants from Belgian sites in accordance with art. 12 of the said law.

For non-Belgian Participating Sites

The Participating Site shall have and maintain in full force and effect during the term of this Trial (and for a reasonable period following termination of the Trial, adequate insurance coverage for other possible damages resulting from the Trial at the Participating Site, as required by local law. Each such insurance coverage shall be in amounts appropriate to the conduct of the services of the Participating Site under this Trial. The Participating Site and Sponsor shall be solely responsible for any deductible or self-insured retention under any such policies.

9.7 Modifications

During the Trial the Sponsor may modify the Trial. It is the Sponsor's responsibility to assess whether a modification is substantial or non-substantial, a change relevant to the the supervision of the Trial, or whether a substantial modification leads to changes in the Trial to an extent that it has to be considered as a completely new clinical Trial.

A "substantial modification" is defined as any change of the Trial which is made after a decision is issued on a previously submitted application and that is likely to 'substantially impact the subjects' safety or rights or the reliability and robustness of the data generated in the Trial. A non exhaustive list of examples of substantial and non-substantial modifications is available in Annex III of the CTR Q&A document in Eudralex

volume 10. Sponsor shall submit the substantial modifications in CTIS which will be evaluated by the CA/EC. A substantial modification may only be implemented if it has been approved by the CA/EC with no prejudice to the right of Sponsor and the Principal Investigator to take urgent safety measures without awaiting prior authorisation. In that case, the event and the measures taken should be reported in CTIS without undue delay but no later than 7 days from the date the measures have been taken.

A "change which is not a substantial modification but relevant to the supervision of the Trial by the CA/EC" shall be permanently updated in CTIS by the Sponsor, in line with article 81(9) of the CTR. An example of such change is an update of sponsor or CRO contact details.

A "non-substantial modification" is any change outside the scope of a substantial modification and irrelevant to the supervision of the Trial. A non-substantial modification should not be notified as such. These changes should be implemented during the next substantial modification. Non-substantial modifications need to be listed and identified as such in the cover letter of the substantial modification application.

9.8 Post-Trial activities (if applicable)

After completing the clinical Trial, participants will not be provided semaglutide. Semaglutide is approved and available to patients on the Belgian market at own expense, after medical advice and prescription as needed. Following study completion, participants will return to and continue routine follow-up within the multidisciplinary obesity clinic according to standard clinical care pathways. Continued multidisciplinary obesity management, including dietary support, psychological support and physical activity support, will remain available independently of continuation or discontinuation of semaglutide treatment.

10 Research Registration, Dissemination of Results and Publication Policy

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every research Trial involving human participants be registered in a publicly accessible database before recruitment of the first participant. The CI is responsible for registering the Trial.

In addition, the CI will fulfil their ethical obligation to disseminate and make the research results publicly available. As such the CI is accountable for the timeliness, completeness and accuracy of the reports. Researchers, authors, Sponsors, editors and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

Publications will be coordinated by the CI. Authorship to publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

11 Intellectual Property

Any know how, inventions, methods, developments, innovations, discoveries and therapies, whether patentable or not, arising from the Trial or made in the performance of the Trial protocol ("Inventions") shall vest in the Sponsor. The Participating Site, its employees and Investigator(s) shall promptly disclose to the Sponsor any such Inventions. Parties have expressly agreed that any and all Trial data as collected and prepared in the performance of the Trial protocol shall be the sole property of Sponsor unless otherwise agreed in the clinical Trial agreement.

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APPENDICES

13 Appendix 1: Clinical Trial protocol history

Original CTP version:	1.1. dated 22/05/2026

Amendment #1:	<CTP version nbr> dated 22/05/2026
Modifications made / Reason for amendment:	
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>

Amendment #2:	<CTP version nbr> dated 22/05/2026
Modifications made / Reason for amendment:	
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>
<protocol section reference>	<describe modifications made>