Dapiglutide for the treatment of obesity (DREAM): a randomised, double-blind, placebo-controlled, investigator-initiated trial

Protocol code number:	The 'DREAM' trial
EU Trial number:	2022-501649-54-00
ClinicalTrials.gov identifier:	*Currently N/A*
Investigational medicinal product:	Dapiglutide 10 mg/ml
Principal investigator:	Filip Krag Knop, MD PhD
Co-investigators:	[redacted], MSc
	[redacted], MD, PhD
Sponsor-investigator:	Center for Clinical Metabolic Research Herlev-Gentofte Hospital Gentofte Hospitalsvej 7, 3rd floor DK-2900 Hellerup
Research location(s):	Center for Clinical Metabolic Research Herlev-Gentofte Hospital Gentofte Hospitalsvej 7, 3rd floor DK-2900 Hellerup
	Gastroenterologist, [redacted] Mavespecialisten Jægersborg Allé 16, 2. sal DK-2920 Charlottenlund
Monitor:	The GCP-unit at Copenhagen University Frederiksberg Hospital Nordre Fasanvej 57, Skadestuevej 1 DK-2000 Frederiksberg
Date:	Wednesday, 04 January 2023

_____

Principal Investigator

Professor Filip Krag Knop, MD PhD

_____

Sponsor-Investigator

Filip Krag Knop, Center for Clinical Metabolic Research

_____

Co-Investigator

Casper Kjærsgaard Nielsen, MSc, PhD-stud

Table of Contents

1. Introduction

Obesity has become a pandemic affecting more than a billion individuals globally[1], and numbers are growing each year[2]. Moreover, obesity is the fifth leading cause of death worldwide[3,4] and increases the risk of diseases such as type 2 diabetes, cancer, sleep apnoea, cardiovascular diseases and non-alcoholic fatty liver disease (NAFLD). Additionally, obesity is associated with psychological problems and reduced quality of life[5–13].

Obesity is a multi-factorial condition arising due to a positive energy balance resulting in excess storage of fat in adipose tissue as well as in other tissues (ectopic fat deposition)[14,15]. Excess storage of fat in insulin-sensitive tissues such as skeletal muscle, liver and adipose tissue results in insulin resistance as well as low-grade systemic inflammation, which in turn exacerbates insulin resistance[16]. Systemic low-grade inflammation and insulin resistance drive the development of many co-morbidities related to obesity. It has become clear that obesity is associated with reduced gut barrier function, i.e., a leaky gut, increasing translocation of microorganisms and their products, such as lipopolysaccharide (LPS), into the bloodstream[17]. This leaky gut seems to contribute importantly to obesity-related systemic low-grade inflammation and, thus, insulin resistance, fuelling the development of co-morbidities related to obesity, including type 2 diabetes, NAFLD with non-alcoholic steatohepatitis (NASH), atherosclerotic cardiovascular diseases and certain cancer forms[18–20].

Despite increasing awareness of obesity and its underlying complex pathophysiology, effective treatments, dually targeting calorie intake and low-grade systemic inflammation, are warranted.

Glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2) are co-secreted from enteroendocrine L cells in response to nutrient ingestion. These peptide hormones act via their distinct receptors, the GLP-1 receptor (GLP-1R) and the GLP-2 receptor (GLP-2R). Besides its glucose-lowering effect, GLP-1 reduces appetite and induces satiety, translating to reduced energy intake. Presently, two GLP-1 receptor agonists (GLP-1RA), liraglutide and semaglutide, are approved for the treatment of obesity[21,22]; and both of these treatments result in loss of primarily body fat mass, translating to improved insulin sensitivity and lowering of the systemic low-grade inflammation originating from excess fat storage. GLP-2R agonism increases perfusion in mesenteric vessels and promotes regenerative function and proliferation of gut epithelial cells resulting in mucosal growth with increased crypt depth, villi height and barrier function[23,24]. Thus, GLP-2R agonism may restore gut barrier function in obesity and thereby diminish systemic low-grade inflammation arising from obesity-associated leaky gut. Despite the obvious fit of GLP-1R and GLP-2R agonism in terms of addressing fundamental aspects of obesity pathophysiology, dual GLP-1R/GLP-2R agonism has yet to be explored as a strategy for the treatment of obesity.

Dapiglutide is 33-amino acid peptide dual GLP-1R/GLP-2R agonist with a C18 acyl modification, which increases the half-life to approximately five days. Dapiglutide is administered

subcutaneously once weekly and is currently under clinical development. In a recently completed phase I trial in normal-weight individuals, 3.5 mg dapiglutide administered once weekly caused a mean 4.3 ± 1.4% body weight reduction following 4 weeks of treatment compared to placebo. Moreover, dapiglutide at various doses ranging from 1.0 to 6.0 mg exhibited a tolerable safety profile, with adverse events (AEs) comprising mild to moderate in intensity and transient in duration. The most frequently observed AEs were nausea and decreased appetite as normally observed with GLP-1RAs[25].

The present protocol is designed to explore the effect of 12-week treatment with once-weekly dapiglutide (4 mg and 6 mg) as compared to placebo, on body weight in individuals with obesity.

2. Aims of the study

This randomised placebo-controlled study will investigate the efficacy of once-weekly subcutaneously administered dapiglutide in obese individuals during a 12-week treatment period.

The primary aim is to evaluate the effects of 4 mg and 6 mg dapiglutide on change in body weight (%).

Secondary and exploratory aims include evaluating the effects of 4 mg and 6 mg dapiglutide on:

- Gut barrier function and blood microbiome
- Systemic inflammation
- Safety and tolerability
- Lean body mass, fat mass, and visceral fat mass
- Patient-reported outcomes (PROs)

3. Hypothesis

We hypothesise that:

1) 6 mg dapiglutide treatment for 12 weeks is superior to placebo treatment for 12 weeks in reducing body weight relative to baseline
2) 4 mg dapiglutide treatment for 12 weeks is superior to placebo treatment for 12 weeks in reducing body weight relative to baseline
3) 6 mg dapiglutide treatment for 12 weeks is superior to 4 mg of dapiglutide treatment for 12 weeks in reducing body weight relative to baseline

4) Dapiglutide will improve gut barrier function (as assessed by circulating levels of LPS-binding protein (LBP))

5) Daspiglutide will reduce low-grade systemic inflammation (as assessed by circulating levels of high-sensitivity C-reactive protein (hs-CRP) and interleukin (IL)-6)

4. Methods

4.1 Study design

This study is an investigator-initiated, proof-of-concept, randomised, double-blind, placebo-controlled, parallel-group, single-centre clinical trial investigating the body weight loss potential of dapiglutide, a dual GLP-1R/GLP-2R agonist, administered subcutaneously once weekly.

Eligible subjects are randomised to one of three treatment arms as illustrated in **Table 1**:

Treatment arm	IMPs	Dose	Pharmaceutical dosage form	Route of administration	Administration frequency
#1	Dapiglutide (10 mg/ml)	4 mg	1 ml solution for injection in vial	Subcutaneously	Once weekly
#2	Dapiglutide (10 mg/ml)	6 mg			
#3	Placebo (n/a)	4 mg			
		6 mg			

Table 1 Overview of treatment arms, dose, usage, route- and frequency of IMP administration.

In total, 54 obese participants with a body mass index (BMI) of ≥ 30 kg/m² are randomised to either treatment with the investigational medicinal product (IMP), being either dapiglutide 4 mg, dapiglutide 6 mg, or placebo for 12 weeks. To ensure blinding, the placebo arm is split between 4 mg and 6 mg placebo, making the randomisation sequence 2:2:1:1. The trial encompasses a 3-week screening period containing a screening visit (V1) to assess eligibility, followed by a randomisation visit (V2) and subsequently a 12-week treatment period concluded with a 4-week follow-up period. The IMP is subcutaneously administered in the abdomen once weekly from week 0 (V2) until week 12 (V14) (**Table 2**). The IMP is initiated at 2 mg once-weekly and up-titrated every third week with 2 mg until the respective trial doses are reached in each arm (**Figure 1**). Hereafter, the participants are kept at the dose level for the remainder of the trial (from week 3 and week 6 for the 4 mg and 6 mg doses, respectively). To reduce dropout in cases of low tolerability of the IMP, the investigator can postpone up-titration or down-titrate if judged necessary for participant retention or safety. The trial schedule will consist of five on-site visits, including screening,

randomisation and a safety follow-up visit (four weeks after end of treatment (EOT)), in addition to a minimum of 10 telephone consultations. Therefore, the maximum trial duration is 16 weeks. For exploratory purposes, participants are invited to participate in a gastroduodenoscopy sub-study obtaining gastric and duodenal biopsies before and after treatment with IMP. A maximum of 7 participants from each treatment arm (total n=21) can participate in this sub-study.

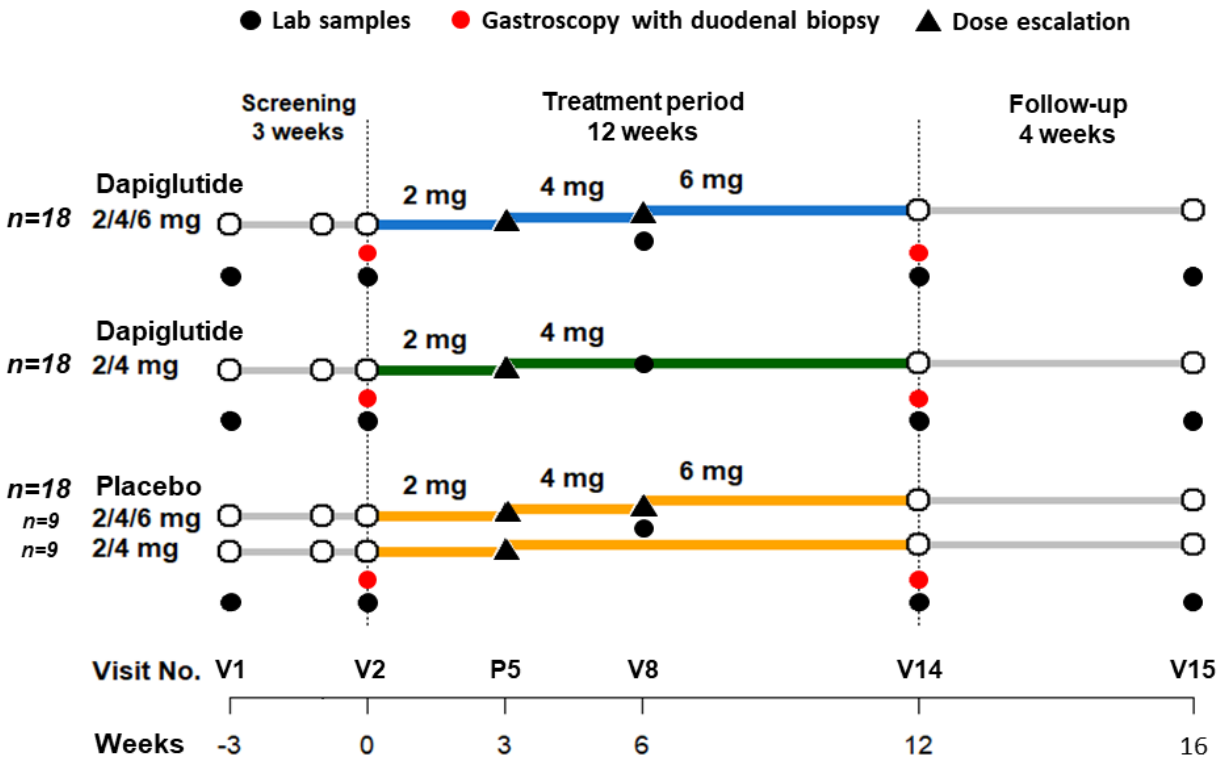

Figure 1. Illustration of the randomised (2:2:1:1) parallel-group, double-blind, placebo-controlled study design in which 54 individuals with obesity will receive either 1) dapiglutide 2/4/6 mg, 2) dapiglutide 2/4 mg, 3) placebo 2/4/6 mg, or 4) placebo 2/4 mg for a period of 12 weeks.

4.2 Flowchart

	Screening	Randomisation	Dose escalation and treatment period											End of treatment (EOT)	End of trial (follow-up visit)
Visit	**V1**	**V2**	P3	P4	P5	P6	P7	**V8**	P9	P10	P11	P12	P13	**V14**	**V15**
Time (weeks)	-3	0	1	2	3	4	5	6	7	8	9	10	11	12	16
Visit window (days)	-21	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±5
General															
Informed consent	x														
Assessment of eligibility criteria	x	x													
Demography	x														
Medical history	x														
Concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Smoking/alcohol	x														
Assessment of childbearing potential	x														
Attend visit fasting		x						x						x	
Gastroduodenoscopy and biopsies[I]		(x)												(x)	
Drug dispensing		x						x							
Drug accountability		x						x						x	
Drug handling instruction	x	x	x	x	x	x	x	x	x	x	x	x	x		
Drug dosing[II]		x	x	x	x	x	x	x	x	x	x	x	x		
Clinical assessment															
Body height	x														
ECG	x														x
Endpoints															
Body weight	x	x						x						x	x
Bioimpedance		x						x						x	x
FibroScan®		x						x						x	x
Waist circumference		x						x						x	x
Blood pressure (systolic and diastolic)		x						x						x	x
Heart rate		x						x						x	x
Biosamples[III]		x						x						x	x
Gut permeability biomarkers		x						x						x	x
Inflammatory biomarkers		x						x						x	x
Leukocytes		x						x						x	x
Lipids		x						x						x	x
Oxidative stress		x						x						x	x

Islet function and incretin		x						x						x	x
Bone biomarkers		x						x						x	x
Plasma PK sample								x						x	x
Blood microbiome		x						x						x	x
Additional blood tests		x						x						x	x
ADA		x						x						x	x
Faecal collection		x												x	
AEs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Patient-reported outcomes															
SF-36		x												x	
IWQOL-Lite-CT[26]		x												x	

Table 2. ADA, anti-drug antibodies; AEs, adverse events; ECG, electrocardiogram; IWQOL-Lite-CT, The Impact of Weight on Quality of Life-Lite Clinical Trials Version; PK, pharmacokinetic; SF-36, The 36-Item Short Form Survey.
[I] Not mandatory for study participation, i.e., optional (provided acceptance from the participant). For feasibility purposes, the gastroduodenoscopy can be conducted up to four days before V2 and V14.
[II] Dosing of IMP occurs supervised either by physical presence at the research center or virtually during the phone visits
[III] All biosamples (i.e. ADA, PK etc.) are collected prior to dosing during V2, V8. If IMP is dosed on the day of the sampling visit, ADA and PK samples are collected before dosing. Samples collected at V14 are approximately one week after the last dosing

4.3 Blinding and randomisation

A total of 54 participants are randomised into three treatment arms (2:2:1:1) (**Figure 1**). A clinical assistant not otherwise affiliated with the study and with experience in producing randomisation lists will generate the randomisation list using https://www.sealedenvelope.com. The placebo arm is divided into two subgroups (4 mg and 6 mg volume-matched placebo) to ensure the double-blind procedure. The randomisation will employ blocks of randomised stratification with respect to sex, thereby securing balanced numbers of males and females in each treatment group (placebo, 4 mg or 6 mg of dapiglutide)[27].

A person not otherwise affiliated with the study is responsible for the treatment allocation and sub-study and has access to the electronic randomisation key and sealed code-break envelopes in case of any medical emergencies. All clinical personnel affiliated with the study have access to sealed emergency envelopes. In such an event, the person breaking the code must complete, sign and date a Code Break notification, reporting the reason for unblinding. If the code is broken, the participant is automatically discontinued from the treatment and the study. Unblinding may be carried out at any time per investigator's discretion.

Participants will always be able to contact study personnel, ensuring contact with the principal investigator in case of an emergency requiring unblinding.

Zealand Pharma A/S will provide IMP (placebo and dapiglutide 10 mg/ml) in indistinguishable vials/cartons.

5. Study population

Fifty-four participants with obesity (BMI of ≥ 30 kg/m²) are included in the study. Dropouts will only be replaced if the dropout rate surpasses 25% in one of the treatment arms. The recruitment process is described in detail in **Section 5.2**.

5.1 Eligibility criteria

5.1.1 Inclusion criteria

- Age 18–75 years
- BMI ≥ 30 kg/m²
- History of at least one attempt to lose body weight

5.1.2 Exclusion criteria

- A self-reported change in body weight ≥ 5% within the last 90 days prior to the screening visit
- Treatment with any therapy, including endoscopic procedures and/or medication (e.g. liraglutide, bupropion/naltrexone and orlistat), intended for weight management within 90 days prior to screening
- Previous, current, or planned (during the trial period) obesity treatment with surgery or a weight loss device < 1 year prior to screening
- Glycated haemoglobin (HbA1c) ≥ 48 mmol/mol
- History of type 1 diabetes or type 2 diabetes
- Treatment with glucose-lowering agents within 90 days prior to screening
- Compromised kidney function (estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2) at screening
- Known liver disease (except for non-alcoholic fatty liver disease) and/or elevated plasma alanine aminotransferase (ALT) > three times the upper limit of normal at screening

- History of acute and/or chronic pancreatitis
- History and/or family history of medullary carcinoma and/or multiple endocrine neoplasia syndrome
- Inflammatory bowel disease
- Any history of colon cancer or intestinal polyps
- Any history of intestinal stenosis
- History of any other cancers (except margin-free resected cutaneous basal or squamous cell carcinoma or adequately treated in situ cervical cancer) unless disease-free state for at least five years
- Uncontrolled thyroid disease as per discretion of the investigators
- Any of the following: myocardial infarction, stroke, hospitalisation for angina and transient ischaemic attack within the last 60 days prior to screening
- Class IV heart failure according to the New York Heart Association
- Any concomitant disease or treatment that, at the discretion of the investigators, might jeopardise the participant's safety during the trial
- Alcohol/drug abuse as per discretion of the investigators
- Known or suspected hypersensitivity to the trial product or related products
- Previous treatment with the trial product
- Administration of an investigational drug within 90 days prior to screening
- Simultaneous participation in any other clinical intervention trial
- Mental incapacity or language barriers that preclude adequate understanding or cooperation, or unwillingness to comply with trial requirements
- Use of GLP-1RA, GLP-2RA, dipeptidyl peptidase 4 (DPP) inhibitors, human growth hormone, somatostatin, or analogues thereof, within three months prior to screening
- Known radiation enteritis or significant villous atrophy, e.g., due to active coeliac disease or inflammatory bowel disease
- Regarding fertile men and women:
    - Women who are pregnant, breastfeeding, intend to become pregnant or are of childbearing potential will not be included in the study
    - Sterilised or postmenopausal women (> 12 months amenorrhoea or females ≥ 60 years of age) can be included

- The following contraceptive methods are considered adequate for study enrolment of male participants: Surgically sterilised or willing to refrain from sexual intercourse from screening and until completion of the follow-up visit, or, if sexually active, condom usage and partner-practised contraception during the trial, i.e., from screening to the last visit

5.2 Recruitment and informed consent procedure

Participants are recruited via advertisement in newspapers and on the internet (including social media, www.forsøgsperson.dk and www.forskningnu.dk), via general practitioners in the Capital Region of Denmark and Region Zealand, and from registries of previous research participants who have agreed to be approached for other studies. Advertisement on social media is in accordance with GDPR, meaning that comments are turned off, and inquiries of study participation from potential participants will be carried out through direct e-mail to the investigator or/and via a link to www.forsøgsperson.dk and www.forskningnu.dk. The recruitment will take place at the Department of Medicine at Gentofte Hospital with recruitment assistance from study group personnel affiliated with different hospital sites in Denmark. Potential participants from the outpatient clinics are asked about their interest in the study and be contacted by telephone or e-mail. According to their preferences, potential participants from previous studies are contacted by telephone or e-mail.

Written information about the study is sent to potential participants, including information about the study, eligibility criteria, legal rights during participation in a biomedical research project and contact information of the principal investigator. Within a week after receiving the written information, potential participants are contacted by telephone to clarify and elaborate on the study information. The telephone call includes details on the purpose of the study and study conduct, potential benefits and risks, reimbursement, protocol compliance, and an estimation of the timeframe of study participation. If the potential participant is still interested in participating, a screening visit (V1) is scheduled. The subject is informed about the right to bring an assessor to the screening visit (V1) and all other trial visits. The screening visit will take place at Center for Clinical Metabolic Research at Gentofte Hospital – in a closed room without any disturbances. Trial information is given only by delegated GCP-trained study personnel. A physician performs the interview and collection of the informed consent during the screening visit. During the screening visit, it will be made clear that the purpose of the meeting is to inquire about potential participation in the study and that written and oral consent to study participation can be withdrawn at any time without further explanation. Trial information is given in terms understandable to the individual participant and will include more details on the purpose of the study, study conduct, potential benefits and risks, reimbursement, protocol compliance and an estimation of the timeframe of study participation. In addition to the trial information, the subject is reminded to

maintain habitual diet and exercise patterns in conjunction with the recitation of the Danish health authorities' recommendation regarding diet and exercise: Eat plant-rich, varied and not too much and exercise (moderate intensity) for 30 minutes a day[28]. During this verbal information, the participant is encouraged to ask questions at any time. When all questions are answered and the participant has a full scope of the trial, the participants are given time to reflect. Reflection time is determined by the participant and can be up to one week. As a default, the subject is given a minimum of 5 minutes alone to reflect and/or discuss the content of the trial with the assessor. Hence, the screening visit cannot take place immediately after the oral information if the potential participant wishes time to reflect. All participants have the right to a minimum of 24 hours of consideration before consenting to trial participation. If the subject declines further reflection time and expresses interest in study participation, written informed consent and formal documents are signed by both parties. Hereafter, the clinical assessments needed for the evaluation of eligibility are conducted (body weight measurement, ECG, blood sample collection etc.). In case of study participation ineligibility, the subject is reminded of the predefined inclusion- and exclusion criteria determining study participation and is thanked for the interest shown in the trial. Throughout the study, any new personal health information obtained is shared with the participant.

6. Endpoints

Primary endpoint			
No. of endpoint	**Endpoint title**	**Time frame**	**Unit**
#1	Change in body weight	From week 0 (baseline) to week 12 (EOT)	%-point

Secondary endpoints			
No. of endpoint	**Endpoint title**	**Time frame**	**Unit**
#2	Body weight reduction ≥ 5%	At week 12 (EOT)	Yes/No
#3	Body weight reduction ≥ 10%	At week 12 (EOT)	Yes/No
#4	Change in fasting serum/plasma concentrations of gut permeability biomarkers	From week 0 (baseline) to week 12 (EOT)	%-point

	• LPS-binding protein (LBP)		
#5	Change in fasting serum/plasma concentrations of inflammation markers • hs-CRP • IL-6	From week 0 (baseline) to week 12 (EOT)	%-point
#6			
Exploratory endpoints			
#7	Body weight reduction ≥ 15%	At week 12 (EOT)	Yes/No
#8	Change in BMI (kg/m^2)	From week 0 (baseline) to week 12 (EOT)	%-point
#9	Change in waist-hip ratio	From week 0 (baseline) to week 12 (EOT)	%-point
#10	Change in waist circumference (cm)	From week 0 (baseline) to week 12 (EOT)	%-point
#11	Change in systolic blood pressure (mmHg)	From week 0 (baseline) to week 12 (EOT)	%-point
#12	Change in diastolic blood pressure (mmHg)	From week 0 (baseline) to week 12 (EOT)	%-point
#13	Change in heart rate (beats per minute)	From week 0 (baseline) to week 12 (EOT)	%-point
#14	Change in fat-free mass as measured by bioimpedance	From week 0 (baseline) to week 12 (EOT)	%-point
#15	Change in total fat mass as measured by bioimpedance	From week 0 (baseline) to week 12 (EOT)	%-point
#16	Change in visceral fat mass rating as measured by bioimpedance	From week 0 (baseline) to week 12 (EOT)	%-point

#17	Change in bone mass as measured by bioimpedance	From week 0 (baseline) to week 12 (EOT)	%-point
#18	Faecal microbiota composition	From week 0 (baseline) to week 12 (EOT)	%-point
#19	Change in fasting serum/plasma concentrations of inflammatory biomarkers • Fibrinogen • Serum amyloid A • Haptoglobin • Soluble cluster of differentiation 14 • Lactate dehydrogenase • IL-1 receptor antagonist, IL-1β, IL-2, IL-8, IL-10, IL-12, IL-17, IL-18, IL-22 • Interferon γ • Resistin • Tumour necrosis factor α • Adiponectin	From week 0 (baseline) to week 12 (EOT)	%-point
#20	Change in fasting serum/plasma concentrations of leukocytes • Total leukocyte count • Neutrophil • Lymphocyte • Basophil • Eosinophil • Neutrophil:lymphocyte ratio	From week 0 (baseline) to week 12 (EOT)	%-point
#21	Change in fasting serum/plasma concentrations of lipid biomarkers • Low-density lipoprotein cholesterol	From week 0 (baseline) to week 12 (EOT)	%-point

	• Very-low-density lipoprotein • High-density lipoprotein cholesterol • Triglycerides • Lipoprotein (a) • Apolipoprotein B • Apolipoprotein C3		
#22	Change in fasting serum/plasma concentrations of islet function and incretin biomarkers • Glucose • HbA1c • Insulin • C-peptide • Insulin sensitivity as assessed by homeostasis model assessment 2 • Glucagon • GLP-1 • GLP-2 • Glucose-dependent insulinotropic polypeptide	From week 0 (baseline) to week 12 (EOT)	%-point
#23	Change in fasting serum/plasma concentrations of bone biomarkers • C-terminal telopeptides of type I collagen • Amino-terminal propeptide of type I procollagen • 25-hydroxy-vitamin D • Parathyroid hormone • Phosphate • Serum calcium • Urinary calcium	From week 0 (baseline) to week 12 (EOT)	%-point

	• Testosterone • Oestradiol		
#24	Change in fasting serum/plasma concentrations of additional blood tests • Haemoglobin • Thrombocytes • Serum albumin • Potassium • Sodium • Creatinine • eGFR • Standard bicarbonate • ALT • Aspartate aminotransferase (AST) • Gamma-glutamyl transferase • Bilirubin • Amylase • Triacylglycerol lipase • Thyroid-stimulating hormone • Fibroblast growth factor 19 • Fibroblast growth factor 21 • α-hydroxy-4-cholesten-3-one • Leptin • Lipocalin 2 • Zonulin • Citrulline • LPS • D-lactate • Fatty acid-binding proteins	From week 0 (baseline) to week 12 (EOT)	%-point

#25	Change in blood microbiome	From week 0 (baseline) to week 12 (EOT)	%-point
#26	Change in FibroScan®-assessed liver steatosis (dB/m)	From week 0 (baseline) to week 12 (EOT)	%-point
#27	Change in FibroScan®-assessed liver fibrosis (kPa)	From week 0 (baseline) to week 12 (EOT)	%-point
#28	Change in Fatty liver index score	From week 0 (baseline) to week 12 (EOT)	%-point
#29	Change in fibrosis 4 score	From week 0 (baseline) to week 12 (EOT)	%-point
Patient-reported outcomes			
#30	Change in the 36-Item Short Form Survey • Physical functioning • Role-physical • Bodily pain • General health • Vitality • Social functioning • Role-emotional • Mental health • Physical component summary • Mental component summary	From week 0 (baseline) to week 12 (EOT)	Score points
#31	Change in IWQOL-Lite-CT • Pain/discomfort domain score • Psychosocial domain score • Total score	From week 0 (baseline) to week 12 (EOT)	Score points
Safety endpoints			

#32	Number of treatment-emergent AEs	From signed consent form (week -3) to follow-up visit (week 16)	Counts of events
#33	Number of serious AEs (SAEs)	From signed consent form (week -3) to follow-up visit (week 16)	Counts of events

7. Gastroduodenoscopies and biopsies

At visit V2 and V14, a maximum of seven participants from each treatment arm are biopsied from the upper small intestine and the gastric ventricle during a gastroduodenoscopy procedure. Six biopsies are collected from prespecified locations in the duodenum and two biopsies from the gastric ventricle near the pyloric antrum. During the gastroduodenoscopies, luminal fluids will also be collected (approximately 2 ml per visit). The analysis of biopsies and luminal fluid samples will include a combination of full- and single cell transcriptomics, microbiome, proteomics, and metabolomics analysis. Also, histological analysis, including villus height, crypt depth and villus height:crypt depth ratio, as well as relevant immunohistochemistry, is performed.

8. Faecal samples

Faecal samples from a private home setting before and after treatment are shipped in envelopes to the lab by the participants. The faecal samples are collected in DNA genotek buffers which enable the samples to be stored at room temperature for 60 days. Faecal samples are analysed by shotgun sequencing enabling species and potential strain identification.

9. Safety and AEs

The IMP supplier (Zealand Pharma A/S) will receive a monthly report of all AEs regardless of seriousness, severity, or presumed relationship to the trial product from time of signing informed consent until the end of the trial. Participants with AEs will receive relevant monitoring, counselling, clinical assessment, laboratory examinations, and/or treatment at investigator's discretion. All AEs are followed-up until the participant is stabilised/recovered.

9.1 Registration of AEs

The investigator is responsible for detection, documentation, recording, and follow-up of all AEs. All AEs occurring after signed informed consent (V1) until completion of the study period are registered (V15), as illustrated in **Table 1**. The participants are instructed to record AEs in a diary in between site visits, and study staff will enquire about AEs in an open-ended and non-leading way during weekly phone visits. All AEs are evaluated for severity and relationship to IMP by the investigator. All types of AEs are recorded in the electronic case report form (eCRF).

9.2 Definition of events

The study complies with the following definitions of events:

- **AE:** Any unwanted medical occurrence in a participant administered the medicinal product, which does not necessarily have a causal relationship with the 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
- **Adverse reaction (AR):** Any harmful and unwanted reaction to a medicinal product regardless of the dose (causality is mandatory)
- **SAE or serious AR (SAR):** An AE or AR that, regardless of dose, results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or leads to a congenital defect or abnormality (planned hospitalisation is not regarded as an SAE) (the principal investigator is responsible for evaluating the causality of all SAEs; in the case of an SAE, the principal investigator is to actively interview the participant regarding this issue)
- **Suspected unexpected SAR (SUSAR)**: A SAR that is unexpected (not consistent with the applicable reference safety information) the expectedness is based on the Reference Safety Information in the current investigator brochure (IB), which means that all SAEs assessed as being related to the trial drug, are considered unexpected and is reported as SUSARs

For this trial, the following events are to be regarded as **AEs of special interest (AESI)** with data collected under a specific eCRF form and within 24 hours of the investigator's knowledge:

- **Liver injury:** Suspicion of liver injury defined as ALT or AST >3 × upper normal limit (UNL) and total bilirubin >2 × UNL, where no alternative aetiology exists (Hy's law); due to the severity of these events, they should always be reported as a SAEs
- **Acute gallbladder disease:** The occurrence of acute gallbladder disease (i.e., gallstones or cholecystitis)

- **Acute pancreatitis:** The onset of acute pancreatitis is defined based on two of the following features
    - Serum lipase and/or amylase activity ≥3 × UNL
    - Abdominal pain (onset severe, persistent epigastric pain typically radiating to the back)
    - Acute pancreatitis characteristics as determined by imaging
- **Neoplasms**: Any event with the confirmation of malignant or non-malignant neoplasm
- **Medication error, misuse and/or abuse:** Any unintended erroneous IMP treatment leading to harm or potential harm of the participant (e.g., wrong unique dispensing number (DUN), wrong administration route, or accidentally higher dose administered than instructed)
- Suspicion of transmission of infectious agents via the IMP
- Overdose of the IMP (defined as administration of a dose and /or frequency and/or duration of dosing higher than the recommended dose, frequency and duration in the protocol)
- Inadvertent or accidental exposure to the IMP

9.3 Assessment of causality and severity

When assessing the causality and severity of an AE, the following definitions are used:

Causality:

- **Probably**: Good reason and sufficient documentation to assume a causal relationship
- **Possibly**: A causal relationship is conceivable and cannot be dismissed
- **Unlikely**: The event is most likely related to aetiology other than the product

Severity:

- **Mild**: No or transient symptoms, no interference with the participant's daily activities
- **Moderate**: Marked symptoms, moderate interference with the participant's daily activities
- **Severe**: Considerable interference with the participant's daily activities, which the participant finds unacceptable. A severe reaction does not necessarily deem the AE as serious, and an SAE are not always severe in nature

The investigator must determine the most appropriate outcome of the AE.

Final outcome:

- **Recovered/resolved**: The participant has fully recovered, or by medical or surgical treatment, the condition has returned to the level observed at the first trial-related activity after the participant signed the informed consent
- **Recovering/resolving**: The condition is improving, and the participant is expected to recover from the event. This term is only applicable if the participant has completed the trial or has died from another AE. This term is, for example, applicable in events of chronic ongoing illness (e.g. cancer)
- **Recovered/resolved with sequelae**: The participant has recovered from the condition but with lasting effect due to a disease, injury, treatment, or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE
- **Not recovered/not resolved**: The condition of the participant has not improved, and the symptoms are unchanged, or the outcome is not known
- **Fatal**: This term is only applicable if the participant died from a condition related to the reported AE. Outcomes of other reported AEs in a participant before he/she died should be assessed as "recovered/resolved", "recovering/resolving", "recovered/resolved with sequelae", or "not recovered/not resolved". An AE with a fatal outcome must be reported as an SAE
- **Unknown**: This term is only applicable if the participant is lost to follow-up

9.4 Reporting of SAEs, SARs or SUSARs

As soon as possible: All SAEs, SARs and SUSARs are reported by the sponsor-investigator as soon as possible (preferably within 24 hours and no later than 7 days) to Zealand Pharma A/S and the database EudraVigilance. Before being reported, all SARs and SUSARs are unblinded by a clinical assistant who is not otherwise involved in the study.

Within 7 days: A life-threatening or a fatal SUSAR is reported by the sponsor-investigator in the database EudraVigilance as soon as possible and no later than seven calendar days after the first knowledge of the life-threatening or fatal SUSAR. Further relevant information must be provided by the sponsor-investigator in the database EudraVigilance within an additional calendar eight days after the initial report.

Within 15 days: In case of a life-threatening or a fatal SUSAR, the investigator is obligated to supply the database EudraVigilance with complementary and relevant information regarding the follow-up actions made by the investigator. All other SUSARs (non-life-threatening or non-fatal)

are reported by the investigator within 15 days after the investigator's first information about the event.

Within 90 days (after study completion): All SAEs, SARs, and SUSARs are submitted to CTIS in yearly reports, and a list of all AEs and ARs are provided in a final report to CTIS within one year after study completion. Sponsor-investigator submits a yearly list that summarises any SARs and SUSARs as well as a report regarding the safety of study participants in the CTIS system.

10. The rationale for the study design and dose justification

Dapiglutide is currently in phase II development and has previously been administered in healthy volunteers with mean BMI of 24.8 kg/m^2 (19.9−27.9), with the highest doses being one weekly dose of 3.5 mg and 3 once-weekly doses of 6.0 mg. The mean body weight loss was 4.27% at the end of the fourth week of treatment, while it was 0.20% in the pooled placebo group. Mean body weight loss following four once-weekly doses of 3.5 mg was 2.35%.

No AEs leading to withdrawal, severe AEs, SAEs, deaths, or AESI occurred. Approximately 60% (17 of 28) of participants dosed with dapiglutide reported a total of 90 AEs, of which the majority were mild (74 events), and the rest were moderate (16 events). Approximately 40% (5 of 12) of participants dosed with placebo reported a total of 5 AEs, which were all mild. All events (except for one event in a placebo participant) were resolved at the end of the trial.

The incidence of treatment-emergent AEs was similar across dose groups for multiple doses of 1.0, 2.25, and 3.5 mg dapiglutide and just slightly higher than with placebo but increased in the highest dose group after escalation to 6.0 mg dapiglutide from the first dose of 3.5 mg. The most common dapiglutide-related AEs across all dose groups were *nausea, abdominal distension*, *dyspepsia,* and *decreased appetite*. All drug-related AEs were recovered/resolved at the end of the trial.

In order to reduce the number of AEs observed with the multiple 6 mg doses, an up-titration cycle every third week by 2 mg is employed in the present trial. Furthermore, 2 mg dose increments and three weeks at each dose level prior to dose escalation are planned. Dose titration has been used previously with other marketed GLP-1R agonists to make higher doses more tolerable.

11. Pharmacokinetic parameters of dapiglutide

In the single ascending dose trial, ZP7570-18144, the pharmacokinetics displayed dose proportionality with a good dose-response relationship for maximal concentration (C_{max}) and area under the curve $(AUC)_{0\text{-}168h}$. Low inter-subject variability was seen. Maximum dapiglutide concentration was reached after a median of 20 to 24 hours, and the half-life was approximately five days.

In the multiple ascending dose trial, ZP7570-18145, dapiglutide exposure of each dose group linearly increased with dose. Peak concentration (T_{max}) occurred around 24 h after dosing, and concentrations declined thereafter. Similar-shaped profiles were obtained from the dose groups. The variability of C_{max} and AUC_{tau} was low within each dose group. Steady-state was reached after the fourth dose in all groups, and the terminal half-life was approximately five days, which is suitable for once-weekly dosing.

12. Trial assessment

12.1 Risk assessment and ethical considerations

A complete list of possible undesirable effects of dapiglutide is provided in the IB[25]. The most frequent undesirable side effects of dapiglutide reported in the human single-dose and multiple-dose study are gastrointestinal-related; nausea, vomiting, abdominal distension, dyspepsia, and decreased appetite[25]. Injection site reactions, including itching and skin rashes, are observed in most studies involving injectable peptides[25]. It is unknown whether the frequency or intensity of the injection site reaction is different with dapiglutide administration. Dapiglutide was not a local irritant at the injection site in studies involving rats and dogs[25]. Headache has been reported in GLP-1RA studies but not in GLP-2RA studies[25]. In clinical trials, headaches are commonly observed in connection with nausea and vomiting.

There is an inherent risk of inducing an ADA response in studies involving modified peptide administration. In the 4-week and 13-week toxicity studies in rats and dogs, immunogenicity was investigated[25]. The studies showed that ADA formation was not associated with altered pharmacokinetics or pharmacodynamic effects. The long half-life of dapiglutide may increase the risk of ADA, but no ADA has so far been detected in human studies with dapiglutide, comprising up to four weeks of dosing.

12.2 Benefit assessment

GLP-1RA therapy has been demonstrated to reduce body weight in obese individuals in several clinical studies[22]. GLP-2RA therapy has been shown to reduce gastrointestinal transit time and gastric secretions and increase absorption of water and nutrients in humans while decreasing inflammatory mediators in diet-obese rodents, indicating that GLP-2RA therapy could work as an anti-inflammatory agent in obesity. After the follow-up visit, all participants are invited to a diet and exercise group counselling. Therefore, it is believed that all participants benefit from trial participation in terms of improving weight-related health. Furthermore, all participants will receive thorough medical assessments, including testing for liver steatosis, ECG, and blood sampling.

12.3 Benefit-risk assessment

The potential benefits from trial participation mentioned above are anticipated to outweigh any risks and/or discomfort associated with trial procedures and administration of dapiglutide. All participants will receive the trial product free of charge. A permanent weight loss after trial participation is not expected.

13. Investigational medicinal products

The investigator is responsible for dispensing the trial product according to the dosage scheme as planned in **Table 1**. The trial products will only be handled by delegated study personnel and trial participants (after careful instruction of administration and handling). A telephone consultation is conducted on the agreed days of dose escalation to ensure correct escalation.

Zealand Pharma A/S will supply - in hard copy - a trial-specific Instructions For Use (IFU) in local language.

The investigator is obligated to document that thorough instructions for the use of IMP are given to the participants in writing and verbally. The investigator will monitor the first administration at the investigation site and subsequently repeat and recap directions for use verbally prior to administration in the participant's home environment and during the telephone consultations. The dose escalations occur three (P5) and six (V8) weeks after randomisation, as illustrated in **Figure 1**. Thereby, 4 mg or 6 mg constitutes a steady-state phase of six weeks from week 6 to week 12. Participants are instructed to return all dispensed vials and cartons to the clinical trial site.

13.1 Syringe for administration

The syringe and needle used is a BD Plastipak™ 1 ml Syringe with detached BD Microlance™ 3 Needle 26 G /13 mm (BD, Holdredge, US).

14. Identification of investigational medicinal product (IMP)

The IMPs listed in **Table 1** are used in this trial. All IMPs are for single-use and are administered by using a single-use syringe with needle. The IMP is supplied by Zealand Pharma A/S. The investigator is responsible for the supply of appropriate syringes with needle and sharps containers for used syringes.

15. Packaging and labelling

The IMP is packaged, labelled and supplied by Zealand Pharma A/S.

The IMP is packaged and labelled in the local language. Labelling is performed according to Annex VI and Good Manufacturing Practice guidelines of the European Commission, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and local laws and regulations.

Dapiglutide and placebo vials are packed and labelled blinded without revealing the treatment allocation. The labels contain no information about the participants. Each dispensing unit (one carton with three vials) will have a trial unique DUN to be used for drug allocation, accountability, and traceability.

Enough IMP for the scheduled number of participants, including a buffer volume, is packed and supplied.

The IMP allocated to the present trial may not be relabelled or reassigned for use outside the trial.

Please see the Trial Material Manual provided by Zealand Pharma A/S for information on the handling of the IMP.

15.1 Storage of IMP

Dapiglutide and placebo must be stored refrigerated at 2-8°C. Storage conditions for the IMP are detailed on the label. The investigator must ensure the availability of proper storage conditions at the site in a secure area with restricted access. The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or by continuous recording using a qualified temperature monitoring system with an alarm function. The temperature should be evaluated and documented weekly on a temperature log.

The investigator will contact Zealand Pharma A/S in case of temperature deviations outside the acceptable range and must have the capacity to ensure correct storage capacity. IMP that has been stored improperly must be set into quarantine and is not be dispensed to any participant before it has been evaluated and approved for further use. The Investigator must take appropriate action to ensure correct storage. The participants receive verbal and written (the IFU) instructions on how to properly handle and store the IMP.

IMP returned from participants can be stored in a dedicated area at room temperature without temperature monitoring.

15.2 Drug accountability

The investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of the trial product, including the date, quantity, batch number and Dispensing Unit Number, and identification of the participant (ID number) who received the IMP.

Any destruction of used/unused IMP must only take place after prior final reconciliation of trial product and written approval by Zealand Pharma A/S.

16. Early termination of the study

For the individual participant, study participation is terminated if

- The informed consent is withdrawn
- In case continued study participation imposes a health risk for the participant as deemed by the investigator
- The event of exceptional circumstances (e.g. some types of SAEs), making it impossible to complete the study

Participants are thoroughly informed about the decision and underlying causes in all cases. In case of early study termination, the participant will still be invited to a follow-up visit four weeks after being discontinued from study participation. Data collected until the point of early study termination are included in the final data analysis.

The Sponsor/Investigator, Zealand Pharma, or a pertinent regulatory authority may decide to terminate the trial or part of the trial at any time if deemed needed.

Conditions that will warrant termination of the clinical trial include, but are not limited to:

- Unacceptable Risk/Benefit-profile
- Administrative reasons
- The discovery of an unexpected, important, or unacceptable risk to the subjects enrolled in the clinical trial
- A decision of Zealand Pharma or Sponsor/investigator to suspend or discontinue investigation of the IMP
- A fatal or life-threatening SUSAR

If a trial is prematurely terminated or suspended, the sponsor/investigator should promptly inform the subjects and assure appropriate therapy and follow-up. Furthermore, the Sponsor/investigator should promptly inform the IEC and Zealand Pharma and provide a detailed written explanation.

If the benefit/risk analysis has changed after the termination of the trial, the new evaluation should be provided to the IEC in case it impacts the planned follow-up of the subjects who have participated in the trial. Necessary actions needed to protect the subjects will be described.

17. Statistical considerations and calculations

17.1 General statistical considerations

Statistical comparisons are made pairwise between all three treatments. The two placebo arms are pooled when analysed. In this small-scale proof of concept study, the primary, secondary, and exploratory analyses target an efficacy estimand corresponding to the hypothetical treatment difference found if everyone in the target population had completed the treatments. In particular, study participants completing < 75% of their assigned treatment are considered as non-completers, and their outcomes at follow-up are treated as missing data. For comparability with other obesity trials, the primary analysis is supplemented with an intention-to-treat analysis which targets the treatment policy estimand, i.e. the de facto treatment difference that is found in a target population where some are able to complete the treatment while other fail due to side effects and lack of effect (**Section 17.3**).

17.2 Baseline characteristics

Baseline characteristics are summarised as number (%) for categorical data, mean +/- SD for normally distributed data, and median (interquartile range (IQR)) for non-normally distributed numerical data.

17.2.1 Primary analysis

The primary endpoint, % change in body weight from baseline to 12 weeks, is analysed using an ANCOVA model including baseline weight, gender, and treatment arm as covariates. Estimated treatment differences are reported as baseline- and gender-adjusted mean differences with 95% confidence intervals.

To control the familywise error rate at the 5%-level, the treatment differences are tested hierarchically in the following order:

1) 6 mg dapiglutide treatment for 12 weeks is superior to placebo treatment for 12 weeks in reducing body weight relative to baseline

2) 4 mg dapiglutide treatment for 12 weeks is superior to placebo treatment for 12 weeks in reducing body weight relative to baseline
3) 6 mg dapiglutide treatment for 12 weeks is superior to 4 mg of dapiglutide treatment for 12 weeks in reducing body weight relative to baseline

Thus, superiority of 4 mg dapiglutide compared to placebo will only be tested if superiority of 6 mg dapiglutide has previously been concluded and superiority of 6 mg dapiglutide compared to 4 mg dapiglutide is only tested if superiority of 4 mg dapiglutide compared to placebo has previously been concluded. A treatment is considered superior to another if the two-sided 95% confidence interval indicates a ratio effect >1 in the appropriate direction.

17.3 Intention-to-treat analysis

For comparability with other obesity trials, the primary analysis is supplemented by an intention-to-treat analysis in which data from all study participants is included regardless of treatment completion. Genuine missing data from dropouts is handled by placebo imputations assuming that dropouts will have similar outcomes as had they received placebo treatment. Besides the different handling of non-completers and dropouts, the %-point change in body weight from baseline to 12 weeks is analysed using an ANCOVA model similar to the primary analysis. Estimated treatment differences are reported as baseline- and gender-adjusted mean differences with 95% confidence intervals.

17.4 Secondary and exploratory analyses

Outcomes for which %-point changes are targeted are assumed to be approximately log-normally distributed and are analysed using a constrained (i.e., baseline-adjusted) linear mixed model with log-transformed outcome as dependent variable, including gender, visit and the constrained visit-treatment interaction as fixed effects, and assuming an unstructured covariance pattern to account for repeated measurements on each study participant. Estimated treatment differences are back-transformed and reported as baseline-adjusted ratio differences in geometric mean bodyweight with 95% confidence intervals. Other continuous scale outcomes are assumed to be normally distributed and are analysed using the same constrained linear mixed model only without the logarithmic transformation. Estimated treatment differences are reported as baseline-adjusted mean differences with 95% confidence intervals. Binary outcomes are analysed with the chi-square test and reported with risk differences and 95% confidence intervals. In case event counts are low, Fisher's exact test is used in place.

Categorical outcomes obtained from the PROs are summarised in frequency tables and evaluated by a Pearson's chi-squared test. In case event counts are low, Fisher's exact test is used in place.

Scores from the PROs are analysed using a constrained linear mixed model similar to that for the normally distributed outcomes. The scoring of the PROs included in the present study is in accordance with published instructions. A minimal clinically important difference (MCID) is defined as[29]

$$MCID = 0.2 \times SD_{Pretreatment}$$

To guard against false positives, the p-values from the secondary and exploratory analyses are adjusted separately and for each treatment comparison, in turn, using the method of Benjamini and Hochberg[30], which controls the false discovery rate. An adjusted p-value <0.05 is considered statistically significant.

17.4.1 Safety endpoints

AEs, SAEs, SARs, SUSARs and AESIs are reported in numbers and percentages. All events are reported regardles of treatment adherence and dropout. No formal statistical tests are performed due to presumed lack of power.

17.5 Missing data

Numbers and reasons for dropout are tabulated for each treatment arm, and descriptive tables are made to compare the characteristics between the dropouts and the completers. Based on similar studies[31], we aim at having a dropout rate of 10% or less. Missing data in the primary analysis is handled by standard multiple imputations. Missing data in the intention-to-treat analysis is handled by placebo imputations. In case of larger than expected or differential dropout, sensitivity analyses based on best case – worst case scenarios are performed for the primary efficacy analysis and the supplementary intention to treat analyses. Missing data in continuous outcomes from the secondary and exploratory analysis are handled implicitly by maximum likelihood estimation in the constrained linear mixed model. Missing data in binary and categorical outcomes is omitted from analyses. In case of larger than expected or differential dropout, sensitivity analyses based on best case – worsts case scenarios are performed for the secondary binary outcomes.

17.6 Sample size calculation

To detect a 5% loss of body weight after 12 weeks of treatment with dapiglutide and with a significance level of 2.5% using a two-sided t-test (unpaired) and with an estimated standard deviation of 4.0% and assuming a dropout rate of 25%, the study requires 54 participants (3 arms × 14 participants + 12 dropouts). The sample size calculation is based on data from a randomised clinical trial evaluating semaglutide vs liraglutide therapy for the treatment of obesity.[22]

Detectable difference	Standard deviation	Significance level	Power	Subjects per arm	Subjects pr arm (with 25% dropouts)
5%	3.6%	2.5%	80%	12	15
5%	3.6%	2.5%	90%	15	19
5%	**4%**	**2.5%**	**80%**	**14**	**18**
5%	4%	2.5%	90%	18	23

Table 3. Power for sample size with a 2.5% significance level

17.6.1 Secondary endpoint power calculation

Considering the key secondary endpoint, body weight reduction ≥ 5%, after 12 weeks of treatment, n=14 completers in each arm will ensure a power of 83% to detect a 50% difference in success rate in favour of dapiglutide compared to placebo based on the two-sided Pearson Chi-square test, assuming a success rate of at most 10% with placebo and a significance level of 5%.

18. Biological materials

Approximately 20-60 ml of blood is collected during fasting visits (V1, V2, V8 and V14), accumulating a total blood withdrawal of 160 to 240 ml over 19 weeks.

In total, a maximum of 300 ml of blood is collected during the trial period (19 weeks), which is considered a non-substantial amount compared to a donation of blood (450 ml at one time).

Faecal samples are collected before- and after treatment

Six biopsies are taken from prespecified locations in the duodenum and two biopsies from the gastric ventricle near the pyloric antrum.

19. Timeline

First participant first visit (FPFV) is expected in Q4 2022. Last participant last visit (LPLV) is expected in Q4 2023. If the study is prematurely terminated, the investigator will promptly inform the study participants and ensure appropriate therapy and follow-up. The sponsor/investigator will further inform the Danish Medicine Agency (DMA), the Danish Medical Research Ethics Committees and Zealand Pharma A/S.

20. Overview of milestones

2022	Q2	Writing protocol
	Q3	Submission of clinical trial application to authorities
		Study site initiation, including delivery of study drug
		Advertisement / pre-screening
	Q4	FPFV
	Q4	Trial conduct
2023	Q1-Q3	Trial conduct / first participant last visit
	Q4	LPLV / trial conduct
2024	Q1	Trial conduct / LPLV
	Q2-Q3	Data analysis / write-up
	Q2-Q3	Reporting, presentation and publication

21. Financial circumstances

This is an investigator-initiated trial initiated by members of the study group who have no financial interest or gains from the results of this study.

22. Funding

This study is an investigator-initiated trial initiated by the Center of Clinical Metabolic Research. In addition to the IMP provided free of charge, a grant of DKK [redacted] from Zealand Pharma A/S funds the entire study. The grant is received as pure support without any kind of obligation to Zealand Pharma A/S. The grant covers the study's entire expenses, including the investigator's salaries, utensils, blood sample analysis, conduction of visits, etc. The value of the grant is transferred to Center of Clinical Metabolic Research, Gentofte Hospital Denmark. The study group members have no financial connection to Zealand Pharma A/S and will have the opportunity to publish and present the results without interference from Zealand Pharma A/S. By contract agreement, Zealand Pharma is notified a minimum of 30 days before submission for publication and can delay by up to 90 days before such submission to ensure against inadvertent disclosure of unprotected trial drug discoveries or confidential information.

23. Financial compensation to participants

The participants are remunerated for documented travel expenses. Likewise, participants are financially compensated for the inconvenience following study participation with DKK [redacted] due to general labour-heavy trial participation. Additionally, the participants receive DKK [redacted] per

gastroduodenoscopy completed. The participants receive remuneration of DKK [redacted] if the participants resign from study participation or are discontinued per the principal investigator's initiative.

24. Logistics

With the exception of the gastroduodenoscopies, all visits are conducted at Center for Clinical Metabolic Research, Herlev-Gentofte Hospital, at Gentofte Hospital Denmark (Sponsor-investigator and Research Site), which accommodates all the practical necessities to carry out the experimental procedures and storage of IMP before dispensing to participants. The gastroduodenoscopies are performed by affiliated gastroenterologist [redacted], MD, who is co-investigator of the trial.

25. Feasibility

The site staff at Center for Clinical Metabolic Research at Gentofte Hospital, University of Copenhagen, has experience from several clinical studies involving treatment and/or investigation of obesity. Moreover, we have a track record in mechanistic research involving GLP-1 and GLP-2 and are experienced in conducting clinical proof-of-concept studies in the field [32–34].

26. Insurance

All participants are covered by the general patient compensation applicable to public institutions ('*Patienterstatningen*').

27. Registration

The trial is conducted in compliance with the protocol. The trial is registered with ClinicalTrials.gov and CTIS and approved by the Danish Medical Research Ethics Committees, the Danish Medicines Authority and the Data Protection Agency. It is carried out under the surveillance and guidance of the GCP unit, University of Copenhagen, Copenhagen, Denmark, in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)-GCP guidelines, as well as EU Regulation No 536-2014 - Annex I. Data, are collected and stored on an approved and secured server of the Capital Region and Region Zealand via an electronic case report file in REDCap (database program). Data are processed in compliance with the General Data Protection Regulation (GDPR) and the Danish Data Protection Act. Results are presented at national and international scientific meetings and synthesised in one or more scientific manuscripts for publication per the CONSORT

2010 statement in international, peer-reviewed scientific journals. Positive, negative, and inconclusive results together with the statistical method, are published as soon as scientifically justifiable. The summary of the clinical trial results will be made available in CTIS no later than a year after end of trial.

28. Research biobank

Centrifuged blood samples (serum/plasma), faecal material and gastroduodenoscopy biopsies are stored in a biobank (-20°C and -80°C freezers) during the study until the expected termination of the study in January 2028. The biobank is equipped with an alarm, and unique identification is needed to access the freezers. Excess biological material are stored in the same biobank. In the case of future research, the biological material is stored in the biobank after study termination (January 2028). If the biological material is used in future research projects, a new application is formulated to the Region Ethics Committee before conducting the new study. As a default, new consent from participants is requisitioned. However, the Region Ethics Committee can give dispensation in this matter. All samples in the biobank are fully anonymised, and all data protection rules are still followed in this period. ADA samples are analysed at the Charles River Laboratories Edinburgh Ltd, Great Britian and dapiglutide concentrations are determined at York Bioanalytical Solutions Limited, Cedar House Northminster Business Park Upper Poppleton, York YO26 6QR. Collection and processing of data in Great Britain will happen in accordance with chapter V of the Regulation (EU) 2016/67

Biobank location: Center for Clinical Metabolic Research, Herlev-Gentofte Hospital.

29. Data

All data are pseudonymised as all participants are given a unique ID number. Registries with unique social security identification number tied to each individual are kept on a safe and secure server. Data in paper are locked behind double-locks and/or electronic case report files using REDCap or in a closed folder at a secure ‘L:drive’ and ‘P:drive’ at Herlev-Gentofte Hospital. Blinded and pseudonymised data can be shared with Zealand Pharma A/S.

29.1 Data location

Data registries are under the supervision of the Danish Data Protection Agency. Data collected during the trials - such as biological material, ECG readings etc. - are archived for a period maximum of 25 years. Furthermore, the investigator allows direct access to source data and documents (including medical records) when monitoring, auditions, and/or inspections of the GCP-unit and/or the Danish Medicines Agency.

30. Publication

The results derived from this study are submitted for publication as one or several manuscripts in relevant high-impact journals. Positive, negative and/or inconclusive results are published, and results shall be uploaded to CTIS as soon as possible after LPLV. The investigator reserves the right to publish all findings related to the study. However, the investigator is obligated to notify Zealand Pharma A/S of any such publication at least 30 days prior to submission for publication to ensure against inadvertent disclosure of unprotected trial drug discoveries or confidential information. In case of a patent application, Zealand Pharma A/S can require a delay of any publication for up to 90 days.

31. Study group

Per 29th of June 2022:

[redacted], MSc, Clinical Metabolic Research, Herlev-Gentofte Hospital, Gentofte

[redacted], MD, PhD, Clinical Metabolic Research, Herlev-Gentofte Hospital, Gentofte

Professor Filip Krag Knop, MD PhD, Clinical Metabolic Research, Herlev-Gentofte Hospital, Gentofte

32. References

1 2022 World Population by Country. https://worldpopulationreview.com/ (accessed Feb 10, 2022).

2 Tremmel M, Gerdtham U-G, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. *Int J Environ Res Public Health* 2017; **14**: E435.

3 Frühbeck G, Toplak H, Woodward E, *et al.* Obesity: the gateway to ill health - an EASO position statement on a rising public health, clinical and scientific challenge in Europe. *Obes Facts* 2013; **6**: 117–20.

4 Global BMI Mortality Collaboration null, Di Angelantonio E, Bhupathiraju S, *et al.* Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet* 2016; **388**: 776–86.

5 Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *Am J Clin Nutr* 2005; **81**: 555–63.

6 Riaz H, Khan MS, Siddiqi TJ, *et al.* Association Between Obesity and Cardiovascular Outcomes: A Systematic Review and Meta-analysis of Mendelian Randomization Studies. *JAMA Netw Open* 2018; **1**: e183788.

7 Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625–38.

8 Dobbins M, Decorby K, Choi BCK. The Association between Obesity and Cancer Risk: A Meta-Analysis of Observational Studies from 1985 to 2011. *ISRN Prev Med* 2013; **2013**: 680536.

9 Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health* 2009; **9**: 88.

10 Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T. Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study. *Arch Intern Med* 2005; **165**: 2408–13.

11 Resta O, Foschino-Barbaro MP, Legari G, *et al.* Sleep-related breathing disorders, loud snoring and excessive daytime sleepiness in obese subjects. *Int J Obes Relat Metab Disord* 2001; **25**: 669–75.

12 Petry NM, Barry D, Pietrzak RH, Wagner JA. Overweight and obesity are associated with psychiatric disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Psychosom Med* 2008; **70**: 288–97.

13 Rajan TM, Menon V. Psychiatric disorders and obesity: A review of association studies. *J Postgrad Med* 2017; **63**: 182–90.

14 Lund J, Lund C, Morville T, Clemmensen C. The unidentified hormonal defense against weight gain. *PLoS Biol* 2020; **18**: e3000629.

15 Piaggi P. Metabolic Determinants of Weight Gain in Humans. *Obesity (Silver Spring)* 2019; **27**: 691–9.

16 Lastra G, Sowers JR. Obesity and cardiovascular disease: role of adipose tissue, inflammation, and the renin-angiotensin-aldosterone system. *Horm Mol Biol Clin Investig* 2013; **15**: 49–57.

17 Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. *Journal of the Endocrine Society* 2020; **4**: bvz039.

18 Fändriks L. Roles of the gut in the metabolic syndrome: an overview. *J Intern Med* 2017; **281**: 319–36.

19 Jeevendra Martyn JA, Kaneki M, Yasuhara S. Obesity-Induced Insulin Resistance and Hyperglycemia: Etiological Factors and Molecular Mechanisms. *Anesthesiology* 2008; **109**: 137–48.

20 Kessoku T, Kobayashi T, Tanaka K, *et al.* The Role of Leaky Gut in Nonalcoholic Fatty Liver Disease: A Novel Therapeutic Target. *International Journal of Molecular Sciences* 2021; **22**: 8161.

21 Dubé M-C, D'Amours M, Weisnagel SJ. Effect of liraglutide on food consumption, appetite sensations and eating behaviours in overweight subjects with type 1 diabetes. *Diabetes Obes Metab* 2020; published online April 6. DOI:10.1111/dom.14050.

22 Rubino DM, Greenway FL, Khalid U, *et al.* Effect of Weekly Subcutaneous Semaglutide vs Daily Liraglutide on Body Weight in Adults With Overweight or Obesity Without Diabetes: The STEP 8 Randomized Clinical Trial. *JAMA* 2022; **327**: 138–50.

23 Drucker DJ, Yusta B. Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. *Annu Rev Physiol* 2014; **76**: 561–83.

24 Baldassano S, Amato A. GLP-2: what do we know? What are we going to discover? *Regul Pept* 2014; **194–195**: 6–10.

25 Zealand Pharma A/S. Investigator's Brochure ZP7570 Ed. 2.0. 2022; published online April 28.

26 Kolotkin RL, Williams VSL, Ervin CM, *et al.* Validation of a new measure of quality of life in obesity trials: Impact of Weight on Quality of Life-Lite Clinical Trials Version. *Clinical Obesity* 2019; **9**: e12310.

27 Kernan WN, Viscoli CM, Makuch RW, Brass LM, Horwitz RI. Stratified Randomisation for Clinical Trials. *Journal of Clinical Epidemiology* 1999; **52**: 19–26.

28 Se her De officielle kostråd - godt for sundhed og klima. Alt om kost. https://altomkost.dk/raad-og-anbefalinger/de-officielle-kostraad-godt-for-sundhed-og-klima/ (accessed June 22, 2022).

29 Sedaghat AR. Understanding the Minimal Clinically Important Difference (MCID) of Patient-Reported Outcome Measures. *Otolaryngol Head Neck Surg* 2019; **161**: 551–60.

30 Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. *Behavioural Brain Research* 2001; **125**: 279–84.

31 Jastreboff AM, Aronne LJ, Ahmad NN, *et al.* Tirzepatide Once Weekly for the Treatment of Obesity. *New England Journal of Medicine* 2022; **0**: null.

32 Andersen A, Lund A, Knop FK, Vilsbøll T. Glucagon-like peptide 1 in health and disease. *Nat Rev Endocrinol* 2018; **14**: 390–403.

33 Hare KJ, Vilsbøll T, Asmar M, Deacon CF, Knop FK, Holst JJ. The Glucagonostatic and Insulinotropic Effects of Glucagon-Like Peptide 1 Contribute Equally to Its Glucose-Lowering Action. *Diabetes* 2010; **59**: 1765–70.

34 Christensen M, Calanna S, Sparre-Ulrich AH, *et al.* Glucose-dependent insulinotropic polypeptide augments glucagon responses to hypoglycemia in type 1 diabetes. *Diabetes* 2015; **64**: 72–8.