

BMJ Open Impact of body weight, low energy diet and gastric bypass on drug bioavailability, cardiovascular risk factors and metabolic biomarkers: protocol for an open, non-randomised, three-armed single centre study (COCKTAIL)

Jøran Hjelmæsæth,^{1,2} Anders Åsberg,^{3,4} Shalini Andersson,⁵ Rune Sandbu,¹ Ida Robertsen,³ Line Kristin Johnson,¹ Philip Carlo Angeles,¹ Jens Kristoffer Hertel,¹ Eva Skovlund,⁶ Maria Heijer,⁷ Anna-Lena Ek,⁷ Veronica Krogstad,³ Tor-Ivar Karlsen,^{1,8} Hege Christensen,³ Tommy B Andersson,^{5,9} Cecilia Karlsson^{10,11}

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JH and AA contributed equally.

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For numbered affiliations see end of article.

Correspondence to

Professor Jøran Hjelmæsæth;
joran.hjelmaseseth@siv.no

ABSTRACT

Introduction Roux-en-Y gastric bypass (GBP) is associated with changes in cardiometabolic risk factors and bioavailability of drugs, but whether these changes are induced by calorie restriction, the weight loss or surgery per se, remains uncertain. The COCKTAIL study was designed to disentangle the short-term (6 weeks) metabolic and pharmacokinetic effects of GBP and a very low energy diet (VLED) by inducing a similar weight loss in the two groups.

Methods and analysis This open, non-randomised, three-armed, single-centre study is performed at a tertiary care centre in Norway. It aims to compare the short-term (6 weeks) and long-term (2 years) effects of GBP and VLED on, first, bioavailability and pharmacokinetics (24 hours) of probe drugs and biomarkers and, second, their effects on metabolism, cardiometabolic risk factors and biomarkers. The primary outcomes will be measured as changes in: (1) all six probe drugs by absolute bioavailability area under the curve (AUC_{oral}/AUC_{iv}) of midazolam (CYP3A4 probe), systemic exposure (AUC_{oral}) of digoxin and rosuvastatin and drug:metabolite ratios for omeprazole, losartan and caffeine, levels of endogenous CYP3A biomarkers and genotypic variation, changes in the expression and activity data of the drug-metabolising, drug transport and drug regulatory proteins in biopsies from various organs and (2) body composition, cardiometabolic risk factors and metabolic biomarkers.

Ethics and dissemination The COCKTAIL protocol was reviewed and approved by the Regional Committee for Medical and Health Research Ethics (Ref: 2013/2379/REK sørøst A). The results will be disseminated to academic and health professional audiences and the public via presentations at conferences, publications in peer-reviewed journals and press releases and provided to all participants.

Strengths and limitations of this study

- The main strength of the present study is its potential to disentangle the short-term (6 weeks) metabolic and pharmacokinetic effects of bariatric surgery per se and calorie restriction (very low energy diet) by inducing a similar weight loss in the two groups.
- Paired tissue biopsies from the gastrointestinal tract and the liver for 'omics' investigations in combination with in vivo drug disposition activity measures from a cocktail of probes will be provided.
- This study establishes a high-quality tissue biobank for global 'omics' or targeted biochemical analyses.
- The explorative study design limits the clinical generalisability of the results.

Trial registration number NCT02386917.

INTRODUCTION

Obesity represents a global epidemic¹ associated with premature mortality and increased risk for type 2 diabetes, cardiovascular disease and cancer.² Weight loss is the primary treatment of obesity and its comorbidities, and even a small weight reduction has beneficial effects on several cardiometabolic risk factors.^{3,4} Weight loss can be achieved by calorie restriction, exercise, pharmacotherapy or bariatric surgery.⁵ Interestingly, the literature indicates that Roux-en-Y gastric bypass (GBP) may have separate, weight loss-independent, beneficial effects on glucose metabolism and type 2 diabetes, for example, improvements

in insulin sensitivity.^{6–8} The mechanisms behind the acute metabolic improvements seen after bariatric surgery remain, however, less clear.⁹ It is also difficult to distinguish the relative contributions of calorie restriction and weight loss from the surgical procedure. The limited human data available has focused on changes in glucose homeostasis, with few studies assessing involved pathways, of most detailed mechanistic studies conducted are done in rodents.^{8 10–13}

It is important to individualise drug doses in order to both obtain an adequate effect and minimise side effects.¹⁴ For most drugs, dose individualisation is performed by dosing per kg total body weight, although this may not necessarily be the best approach.¹⁵ In patients with morbid obesity, systemic clearance of a cytochrome P450 3A (CYP3A) substrate was found to be similar, while oral bioavailability and volume of distribution were higher compared with patients with normal weight.¹⁶ However, other studies indicated that drug clearance might be deviant in patients with severe obesity.^{17–19} A recent study demonstrated a significant inverse correlation between body mass index (BMI) and protein expression of CYP3A and oral clearance of another CYP3A4 substrate.²⁰ Hence, subjects with severe obesity might be at risk of drug overexposure.²¹ The mechanisms behind the altered expression and activity of this CYP enzyme are unknown, but it has been hypothesised that changed inflammatory state^{22–24} as well as hepatic dysfunction may be involved.²⁵

Several, but not all, bariatric surgery techniques reduce the absorptive surface area by bypassing parts of the intestine.²⁶ Accordingly, bariatric surgery may affect the absorption rate and bioavailability of a range of drugs.^{27–31} Previous studies have shown that the bioavailability of atorvastatin is increased early (months) after surgical reduction of the intestinal surface area.^{32 33} It might be speculated that the net effect of bypassing the metabolic most active parts of the intestine results in this, at a first glance, contradictory effect. Interestingly, an adaptive process in the intestine seems to normalise the bioavailability over a longer time span.³⁴ Even though the literature is sparse and based on small studies, reduced uptake early after intestinal bypass has been shown for drugs substrates of other intestinal enzymes.^{35 36}

Oral bioavailability of drugs is restricted by a variety of transporters and metabolising enzymes in both the enterocytes and hepatocytes. Genetic, environmental and disease-related factors induce variations in expression and activity of these proteins. Genotypic variations can easily be assessed from a single blood sample, but in order to investigate the phenotypic variation, specific probe drugs have to be used in vivo. Several approaches using a cocktail of probe drugs (targeting different CYP enzymes and drug transporters) have been described.³⁷

The present study was designed to disentangle the short-term (6weeks) metabolic and pharmacokinetic (PK) effects of GBP and a very low energy diet (VLED)

by inducing a similar weight loss in the two groups. It will include repeated cocktail investigations of a set of key drug metabolising enzymes and transporters restricting bioavailability for many drugs. The design allows revealing PK changes as well as mechanisms of metabolic changes specifically induced by GBP and calorie restriction per se. Combining the determination of in vivo CYP enzyme and transporter activities with protein expression and ex vivo CYP activity of the same proteins is a powerful tool for further elucidation of the mechanisms of body weight change, GBP and calorie restriction per se on the disposition of drugs, and it allows for improved in vitro–in vivo extrapolations in the future.^{20 37 38} A more detailed description of the study rationale and evidence gap to fill is shown in the online supplementary table 1.^{9 10 20 33 34 39–51}

Study objectives

The primary objectives of this study are related to (1) drug bioavailability and disposition and (2) metabolism, cardiometabolic risk factors and biomarkers.

Drug bioavailability and disposition

- The study aims to investigate the relationship between body composition and the liver/intestine expression and activity of proteins (drug metabolising enzymes, transporters and regulatory factors) important for drug bioavailability and disposition in the range from normal body weight to morbid obesity cross-sectional in three study groups: patients undergoing cholecystectomy, GBP and VLED.
- The study aims to compare the short-term (6weeks) and long-term (2years) effects of GBP and a VLED, with a similar 6-week weight loss, on bioavailability and PKs of probe drugs and biomarkers (and adjoining protein expressions) for CYP1A2, CYP2C9, CYP2C19, CYP3A, P-glycoprotein (P-gp) and organic anion transporting polypeptide 1B1 (OATP1B1).

Metabolism, cardiometabolic risk factors and biomarkers

- The study aims to compare the three study groups (GBP, VLED and cholecystectomy) at baseline with respect to body composition, cardiometabolic risk factors and metabolic biomarkers.
- The study aims to compare the short-term (6weeks) changes in glucose metabolism, blood pressure, blood lipids and body composition of similar weight loss (GBP vs VLED), and long-term effects (2years) of GBP and VLED on body composition, cardiometabolic risk factors and metabolic biomarkers.

The secondary objectives are:

- To compare the short-term and long-term (6weeks - 2years) effects of GBP and VLED on physical activity, energy expenditure, health-related quality of life, anxiety/depression, eating behaviour and obstructive sleep apnoea.
- To assess the relation between proteins and nucleotides at all investigated sites (biopsies and blood).

- c. To perform an in-depth analysis of the correlation between CYP protein expression in vivo and ex vivo CYP activity in jejunum and liver biopsies sampled from the same site in each of the GBP patients, as well as liver biopsies from the cholecystectomy patients.
- d. To investigate the impact of inflammation, gut microbiota/antimicrobial peptides, proteins/peptides, nucleotides and internal body time on cardiometabolic diseases, signalling pathways and PK parameters.
- e. To assess differences in signalling pathways in patients with type 2 diabetes, impaired fasting glucose and normal glucose levels on the protein/peptide, nucleotide, metabolite, lipid and bile acid level with the aim to reveal mechanisms of importance for cardiometabolic diseases.
- f. To investigate differences in signalling pathways in persons with a wide range of BMIs and in patients undergoing similar weight loss by VLED as opposed to GBP with the aim to further elucidate mechanisms important to health and disease.

METHODS AND ANALYSIS

Design and setting

This open, non-randomised, three-armed, single-centre study is performed at a tertiary care centre (Morbid Obesity Centre, Vestfold Hospital Trust) in Norway. The study was designed to follow the current routine treatment procedures at the centre with the exception of the 6-week VLED. The investigations are performed in patients scheduled either for cholecystectomy, or weight loss with GBP or VLED based on clinical indications, and the treatment procedures are not influenced by the present protocol (figure 1).

Patient and public involvement

The development of the research questions and outcome measures were not informed by patients' priorities, experience or preferences. Participants will be sent a summary of the trial findings when the main article is published, and if appropriate, the results will be communicated to policymakers and commissioners of weight management

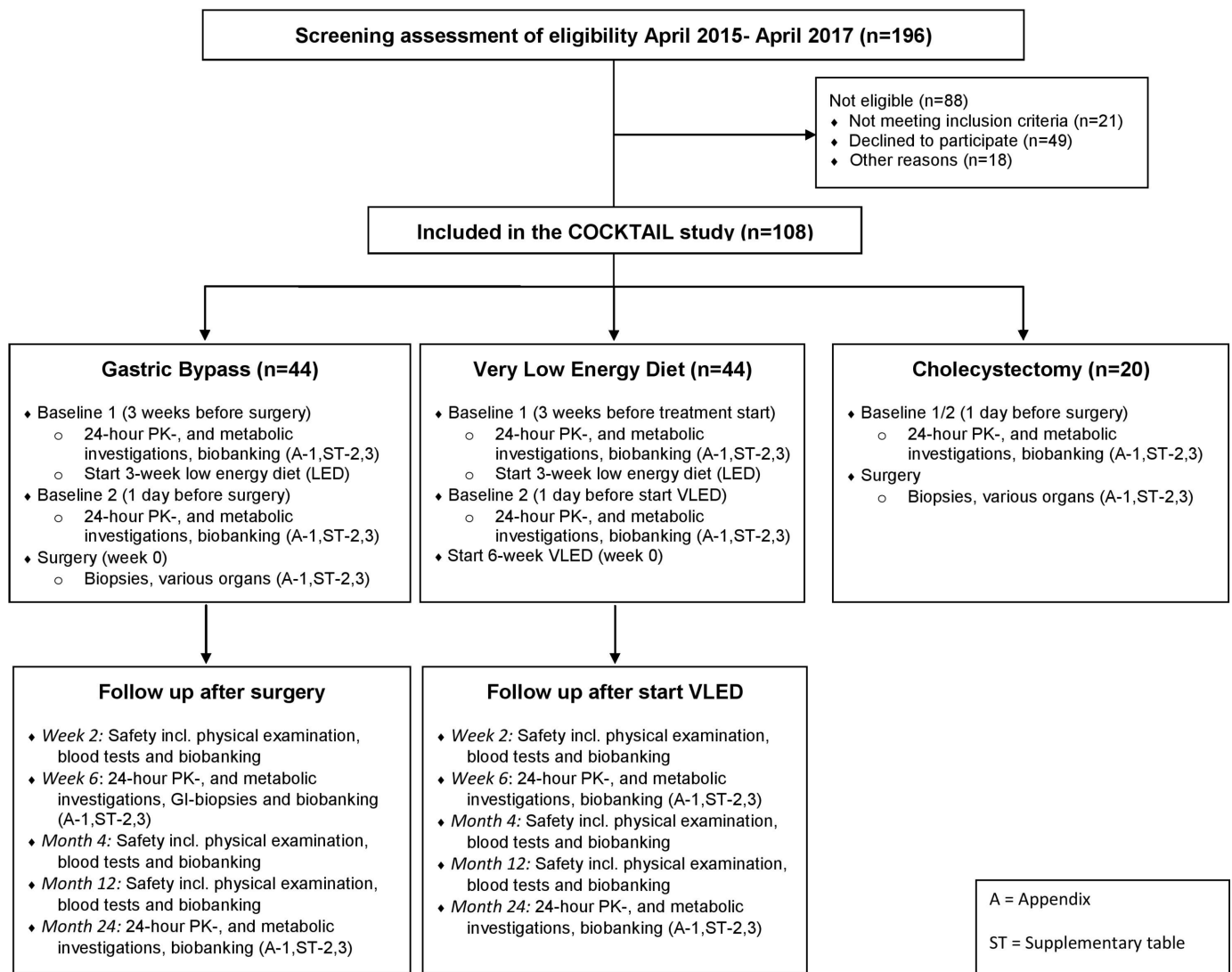


Figure 1 Participant flow from screening to last follow-up. PKs, pharmacokinetics; VLED, very low energy diet.

services through briefing papers summarising the main findings.

Patient selection and recruitment

Consecutive patients scheduled for surgical (GBP) or medical (VLED) weight loss treatment as well as patients scheduled for cholecystectomy were contacted by telephone or face to face at the centre by a project researcher who informed about the study. Patients interested in participation were provided with an invitation letter with detailed information on what taking part in the study would entail, and a time for appointment for screening examination was suggested. At screening, patients were assessed according to the eligibility criteria. Informed consent was obtained before any protocol determined activities, according to the Declaration of Helsinki and Good Clinical Practice (GCP). Patients and the informing physician signed the patient information; original copy was stored at the centre, and the patient received a copy. The patients were also informed that they are covered by liability insurance and that they are allowed to withdraw from the study at any time without giving any reason for doing so.

Inclusion criteria

- ▶ Willing and able to give informed consent for participation in the study.
- ▶ Scheduled for GBP, VLED or cholecystectomy.
- ▶ BMI ≥ 18.5 kg/m².
- ▶ Aged 18 years or above.
- ▶ Able and willing to donate biopsies, perform 24 hours PK investigations and other assessments as required by the clinical study protocol.
- ▶ Stable body weight (<5 kg self-reported weight change) during the last 3 months before inclusion.

Exclusion criteria

- ▶ Concomitant treatment with medications and/or other substances that may influence the cocktail drug PKs such as grapefruit juice, Seville oranges, Pomelo juice, St. John's wort, nicotine and coffee/tea in close approximation to the investigations.
- ▶ Bradycardia, Wolff-Parkinson-White syndrome and atrioventricular block 2–3.
- ▶ Electrolyte disturbances (particularly hypokalaemia or hypomagnesaemia).
- ▶ Estimated glomerular filtration rate (GFR) <30 mL/min/1.73 m².
- ▶ Blood donations the last 4 months before inclusion.
- ▶ Previous bariatric or upper gastrointestinal surgery.
- ▶ Taking glitazones, insulin or sulfonylureas.
- ▶ Pregnancy (checked with HCG in urine at screening) and breastfeeding mothers.
- ▶ Known hypersensitivity (including allergy) to drugs included in the cocktail and/or local anaesthesia.
- ▶ Taking anticoagulants with associated risk in combination with biopsies.
- ▶ Suspected non-compliance with regards to visits and/or diet.

Participant flow and follow-up

A total of 196 patients were screened, out of whom 88 patients were excluded, leaving 108 patients to be included in the study (figure 1 and supplementary appendix 1). After inclusion, both weight loss groups were subjected to a 24-hour PK cocktail investigation (baseline 1), and both groups started a 3-week low energy diet (LED; 1200 kcal/day) directly after. At the 3-week follow-up (baseline 2; day before surgery for GBP group), the 24-hour PK cocktail investigation was repeated. A third group, patients scheduled for cholecystectomy, was subjected to a 24-hour PK cocktail investigation the day before surgery.

The day after the second PK investigation (week 0, baseline 2), VLED patients started the 800 kcal/day diet, while GBP patients were subjected to surgery, and biopsies were obtained from different tissues. The cholecystectomy patients were also subjected to surgery and biopsies the day after their 24-hour PK cocktail investigation. This group did not undergo any further investigations or follow-up. The VLED and GBP groups were followed with 24-hour PK cocktail investigations at the 6-week follow-up, which also will be repeated at the 2-year follow-up. Intestinal biopsies are obtained at the same location in the intestine as during the GBP surgery in these patients at both the 6-week and 2-year follow-ups. Biobanking of samples was, in addition to the visits mentioned above, also performed in both groups at the safety visits (2-week, 4-month and 1-year follow-ups, see online supplementary appendix 1).

During the first 9 weeks of the study (including the 3-week run-in LED diet), patients were closely followed and motivated by the clinical nutrition team to ensure a similar weight loss between GBP and VLED groups. Between the 6-week and the 2-year follow-ups, the patients were offered individually tailored follow-up by the dietitian or other members of the multidisciplinary team at the outpatient clinic.

Sample size

This study has a number of primary study objectives, and the literature on bioavailability after bariatric surgery generally includes very small samples.^{36 52–57} Based on previous studies,^{20 34} we decided to base the sample size calculation on midazolam oral bioavailability in the intervention groups. In order to evaluate the change in midazolam bioavailability from before to 6 weeks after GBP/VLED with an 80% power and a 5% significance level, assuming a bioavailability ratio (6 weeks:baseline) of 1.4 in the GBP group and no change in the VLED group and a SD of 0.5 in both groups,^{20 34} at least 25 patients should be included in each group. Due to the explorative nature of the present protocol, an additional number of patients were included in order to ensure relevant assessments of other outcome variables. Hence, a total of 80 patients were planned to be included in the GBP (n=40) and in the VLED (n=40) groups, and we aimed to substitute premature withdrawals as far as practically possible. The cholecystectomy control group was planned to consist of

20 patients, based on best guess since no previous data were available.

Interventions and biopsy procedures

Gastric bypass

A routine laparoscopic GBP was performed by implementing an antegastric antecolic Roux-en-Y configuration with an omega loop.⁵⁸ Standard port placement was applied with four bladeless trocars and a Nathanson liver retractor (Cook Medical). All stapling was performed using a linear stapler. The pouch was created by stapling the stomach horizontally from the minor curvature and vertically to create a gastric pouch of about 25 mL. The gastrojejunostomy was created using a 45 mm stapler and completed with a running suture. The biliopancreatic limb was 60 cm. The omentum was not transected routinely. The entero-enteric anastomosis was created using a side-to-side technique, using a 45 mm stapler and completed with a running suture. Liver, fat and muscle biopsies were taken at the beginning of the procedure to maximise time to monitor haemostasis.

Cholecystectomy

A standard four-port laparoscopic cholecystectomy was performed with the optical port inserted supraumbilically.

Biopsies

Subcutaneous fat biopsies were obtained in the morning of every visit on all patients. This was done by aspiration through a 14G×3–1/4' G needle, with manual suction on a 20 mL syringe. Infiltration anaesthesia, lidocaine 10 mg/mL with epinephrine five microg/L, was used.

True-cut biopsies of liver, visceral fat and abdominal muscle were collected from all patients undergoing cholecystectomy or GBP at the beginning of each procedure to ensure safe monitoring for bleeding. Bipolar diathermia was used for haemostasis on the cut surface, after the biopsy was taken. A section of the jejunum, where the anastomosis was placed, was also sampled from the GBP patients.

Pinch biopsies of intestinal mucosa in the gastric ventricle, jejunum and ileum were obtained from all GBP patients. This was performed at the moment the intestines were opened for making the anastomoses. Biopsies of the gastric ventricle and jejunum will be repeated with the same pinch technique, at the same site in the intestine, by endoscopy at 6 weeks and 2 years after surgery.

Calorie restriction (interventions)

Low energy diet

The LED diet aimed for an energy intake <1200 kcal per day during the 3-week run-in period in both intervention groups (week -3 to 0). It was based on whole-grain crisp bread (Wasa, Barilla Norge AS, Hamar, Norway) combined with low-fat, high-protein products for three of four daily meals. The fourth meal (dinner) consisted of a specified amount of fish, poultry or lean meat combined with vegetables, potatoes, rice or pasta. Participants were instructed to drink at least 1.5 L of water or energy-free

liquid per day. Supplement of one daily multivitamin and mineral tablet was recommended (Nycoplus Multi, Takeda A/S, Asker, Norway). Additionally, participants could eat an unlimited amount of vegetables and one fruit per day.

Very low energy diet

The VLED diet aimed for an energy intake <800 kcal per day during additional 6 weeks after the completion of the LED (weeks 0–6) implementing the same dietary approach as the LED but with more limited amounts of all food items including vegetables.

GBP calorie restriction

During the first postoperative week, only liquid meals consisting of protein-enriched soups and dairy products every second or third hour through the day were prescribed, and in the second and third postoperative week, a VLED with high-protein, low-fat mashed foods were included. During weeks 4–6, the surgical patients were advised to use the VLED.

Supplementary vitamins and minerals

Standard vitamin and mineral supplementation after GBP surgery were prescribed (two multivitamin/mineral tablets daily (Nycoplus Multi, Takeda A/S, Asker, Norway), two chewable vitamin D/calcium tablets taken morning and evening (each containing 10 µg D₃/500 mg calcium carbonate, Calcigran Forte, Takeda AS, Asker, Norway), iron, 100 mg ferrous sulfate for fertile women or if needed (Duroferon, ACO HUD AB, Väsby, Sweden) and vitamin B₁₂ given intramuscularly 1 month after surgery and thereafter every third month (1 mg Vitamin B₁₂ Depot, Takeda AS, Asker, Norway)).

Schedule of 24-hour PK investigations and measurements

For detailed schedule, see online supplementary table 2. In short, patients are to withhold caffeine-containing beverages from 2 days before the investigation and to start food and drug fasting from 22:00 the day before the investigation. At 07:30, patients meet at the laboratory for baseline blood sampling followed by urine and faeces sampling, subcutaneous adipose tissue biopsies and administration of the cocktail of probe drugs. Blood and urine samples are frequently obtained over the next 12 hours, and the patients will also come back to the laboratory for 23 and 24 hours samples the following day.

The drug cocktail consists of: caffeine (100 mg, oral), CYP1A2; losartan (25 mg, oral), CYP2C9; omeprazole (20 mg, oral), CYP2C19; midazolam (total dose 2.5 mg; 1.5 mg oral at baseline and 1.0 mg intravenous after 4 hours), CYP3A; digoxin (0.5 mg, oral), P-gp; and rosuvastatin (20 mg, oral), OATP1B1.

Sampling and storage procedures of biological material (blood, urine, faeces and biopsies) are shown in the online supplementary table 3. The samples of biological material will be collected in accordance with the schedule of assessments and procedures during the PK investigations (online supplementary appendix 1).

Laboratory methods

Cocktail probe drug (and metabolite) concentrations and other biomarkers (including cytokines) will be analysed in plasma and urine with validated methods based on at that time current guidelines from the US Food and Drug Administration and the European Medicines Agency.

Clinical chemistry analyses will be analysed in serum/plasma, primarily at the Department for Medical Biochemistry at the Vestfold Hospital Trust. They will always include: electrolytes (Na⁺, K⁺ and Ca²⁺), creatinine, ALP, ASAT, ALAT, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, free fatty acids, fasting glucose, fasting insulin (average of two samples obtained 15 min apart), HbA_{1c}, haematocrit and haemoglobin.

Genetic and nucleotide analyses

DNA will be extracted from EDTA whole blood samples throughout the study in VLED and GBP groups (online-supplementary table 4). Approximately 20 µg of DNA will be extracted from each sample. Next-generation sequencing will be applied to capture different types of genetic variation (eg, single nucleotide polymorphisms, copy number variations, whole exons and intergenetic regions and epigenetic modifications) in genetic regions relevant for the probe drug PK, obesity, obesity-related diseases and cardiometabolic status.

Analyses of relevant nucleotide expressions and epigenetic analyses in biopsies and potentially urine will be determined with semiquantitative RT-PCR methods or other appropriate methods.

Protein, metabolite and biomarker analyses

Analyses of biopsy samples will be performed by state of the art methodology (eg, quantitative global and targeted LC-MS/MS analyses or similar). Analyses of lipids, bile acids and metabolites may be conducted with state of the art methodology.

High sensitive C reactive protein, interleukin-6 and insulin will be analysed with commercial ELISA kits.

Microbiota analyses

An external collaborator will perform analyses of microbiota in faeces samples. Also, analysis of antimicrobial peptides in the jejunum is planned.

CYP activity ex vivo

Analyses of CYP activities will be performed in jejunum and liver biopsies from the GBP patients and in liver biopsies from the cholecystectomy patients obtained at the time of surgery. Hepatic and intestinal microsomes will be prepared, and activities of seven CYP enzymes (CYP3A, CYP2C9, CYP2C8, CYP2D6, CYP2B6, CYP1A2 and CYP2C19) will be assessed by incubation with selective probe drugs followed by LC-MS/MS quantification of metabolite formation.

Internal body time

Samples for assessments of internal body time are obtained and will be analysed by validated LC-MS/MS methods.

Registration of questionnaire-based data on patient-reported outcome measures

All collection of questionnaire-based data including health-related quality of life (SF-36,^{59 60} IWQOL-lite,⁶¹ OWQOL and WRSM,^{62 63} anxiety/depression (HADS^{64 65}) and eating behaviour (TFEQ^{66 67}) will be web based by the use of SurveyXact, an internet-based survey system. Patients will be guided into a quiet office at the study centre and asked to fill in the questionnaires (online supplementary table 5). The completion time is estimated to about 45 min.

Registration of physical activity

Physical activity will be monitored by use of an accelerometer (version GT3X+ from ActiGraph, LLC, Pensacola, Florida, USA). The accelerometer provides an objective measure of overall physical activity and will be used the first week in the LED period as well as week 6 after GBP or start of the VLED period, respectively, and again at the 1-year and 2-year follow-up investigation.

Food records

Participants will be asked to record all foods and beverages consumed during a 4-day period (three week days and either Saturday or Sunday) at the following time points: the first, third and sixth week after GBP surgery, and at start of the VLED period, respectively. At the 2-year follow-up, habitual dietary intake in both groups will be assessed by a structured interview performed by registered dietitians, using an optically readable food frequency questionnaire (Department of Nutrition, University of Oslo, Oslo, Norway).

Measurements

The online supplementary appendix 1 shows a summary of the measurements collected.

Sociodemographic characteristics

Age and sex were registered.

Medical history

Medical history, menopausal status, alcohol drinking habits, nicotine use, concomitant drugs (including dietary supplements and OTC drugs like omega-3, St. John's wort and similar substances), possible food/drink interactions, changes in physical activity and/or diet the last 3 months and comorbidities that might affect drug bioavailability were recorded.

In addition, obstructive sleep apnoea was assessed with ApneaLink.

Anthropometric measurements

Body weight was recorded with patients wearing light clothing to the nearest 0.1 kg using an electronic scale

(Inbody 720, Body Composition Analyzer, Biospace, Korea). Height was measured to the nearest 1 cm with a wall mounted measuring tape (Soehnle Professional 5002.01, Backnang, Germany) with patients wearing no shoes. BMI is calculated as weight in kilograms divided by the square of the height in metres. Waist circumference and hip circumference were measured with a stretch-resistant tape parallel to the floor and midway between the 12th rib and the iliac crest, and around the widest portion of the buttocks, respectively. Bioelectrical impedance measures were collected using the Inbody 720, Body Composition Analyzer (Biospace, Korea).

Withdrawals, retention and premature study termination

Patients are free to withdraw at any time in accordance with GCP. Premature withdrawals will lower the power of the study, and therefore each withdrawn patient was substituted when possible. In cases of unnatural high Adverse Event (AE) frequencies as compared with the standard clinical practice the Steering Committee will re-evaluate if it is ethically possible to continue the study.

Analysis plan

Primary outcomes

Drug bioavailability and disposition

- Short-term (6weeks) and long-term (2years) changes in absolute bioavailability (AUC_{oral}/AUC_{iv}) of midazolam (CYP3A4) and systemic exposure AUC_{0-last} , or drug: metabolite ratio, as appropriate, for the other probe drugs and endogenous CYP3A4 biomarkers, after GBP and VLED, respectively.
- Baseline expression, genotypic variation and activity data of the drug metabolising, drug transport and drug regulatory proteins in biopsies from ileum, liver, visceral fat, subcutaneous fat and skeletal muscle in the GBP and cholecystectomy groups.

Metabolism

- Short-term (6weeks) changes in cardiovascular risk factors such as fasting glucose, HbA1c, insulin sensitivity, blood pressure, blood lipid levels, total body fat, BMI, waist and hip circumference and measured cardiometabolic biomarkers and long-term (2years) changes in fasting glucose, HbA1c, insulin sensitivity, blood pressure, blood lipid levels, total body fat, BMI, waist and hip circumference, sleep apnoea and cardiometabolic biomarkers in the GBP and VLED groups.
- Cardiovascular risk factors such as fasting plasma glucose, HbA1c, insulin sensitivity, blood pressure, blood lipid levels, total body fat, BMI, sleep apnoea and cardiometabolic biomarkers (cross-sectional comparison of participants undergoing GBP, VLED and cholecystectomy at baseline).

Secondary outcomes

- Changes in health-related quality of life (SF-36, IWQOL-lite, OWLQOL and WRSM), anxiety/

depression (HADS), eating behaviour (TFEQ), physical activity (accelerometer) and total energy expenditure within the GBP and VLED groups from baseline to the 6-week, 1-year and 2-year follow-ups, and changes in obstructive sleep apnea (ApnelaLink) from baseline to the 6-week, and 2-year follow-ups.

- Baseline characteristics and changes after intervention in proteins/peptides, nucleotides, metabolites, lipids, bile acids and other metabolic/inflammatory/signalling pathways/internal body time parameters in plasma and tissue samples from all patients.
- Short-term (6weeks) and long-term (2years) changes in the expression, nucleotide sequence and activity data of the drug metabolising, drug transport and drug regulatory proteins in biopsies from:
 - The gastric ventricle and jejunum in the GBP group.
 - Subcutaneous adipose tissue in the GBP and VLED groups.
- CYP protein expression and microsomal CYP activity measured by specific activity *ex vivo* in microsomes from intestinal and hepatic biopsy material from each of the GBP patients.
- Genetic variants and epigenetic alterations in genes encoding relevant proteins with regards to obesity and diabetes status.
- Gut microbiota in the three groups at baseline and in the GBP and VLED groups over time and the association with cardiometabolic disease signalling pathways and PK variables.
- Internal body time in the three groups at baseline and in the GBP and VLED groups over time and the association with cardiometabolic disease signalling pathways and PK variables.
- Changes in urine composition predicting development of urine stones in the GBP and VLED groups.
- Changes in urine metabolomics in GBP and VLED groups over time.

Statistical analyses

Repeated measurements over time (2 years, four time points) will be compared between the GBP and VLCD groups by linear mixed models, with the primary endpoints after 6 weeks and 2 years. Additional sensitivity analyses will include analysis of variance (ANOVA) for repeated measurements both applying an intention to treat principle imputing missing observations by multiple imputation techniques and by a per protocol analysis. Linear regression will be applied to assess associations between clinical variables as well as different protein expressions and other continuous variables.

A descriptive analysis of the body composition, glucose metabolism, cardiovascular disease and metabolic biomarkers will be performed between the three groups at baseline, including association analyses. Simple and multiple linear regression models will explore the association with clinical variables in the study.

In the first secondary endpoint, the group effect on health-related quality of life, anxiety/depression, eating

behaviour and obstructive sleep apnoea will be assessed by ANOVA for repeated measurements. Other secondary endpoints will be tabulated and analysed with appropriate methods.

Time schedule

Inclusion first patient: 15 April 2015.

Inclusion last patient: 31 May 2017.

Recruitment time: approximately 2 years.

Follow-up: up to 2 years for each patient.

End of study: LPLV, approximately 4 years after study start (2 years after last patient included), May–June 2019.

Organisation

The COCKTAIL study is a collaboration between the Morbid Obesity Centre, Vestfold Hospital Trust, Norway (sponsor), the School of Pharmacy, University of Oslo, Norway and AstraZeneca Gothenburg, Sweden.

Trial Steering Committee (TSC)

The TSC includes seven persons, two members from each of the two Norwegian collaborating groups (Vestfold Hospital Trust and University of Oslo, Norway) and three members from AstraZeneca Gothenburg, Sweden and is led by Professor Anders Åsberg (Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway). The principal investigator is Professor Jøran Hjelmæsæth (Vestfold Hospital Trust). The TSC will provide oversight of all matters relating to participant safety. Due to the low risk nature of the COCKTAIL study and that it is a pragmatic open-label trial, the TSC also has the role of the Data Monitoring Committee. However, there are no early stopping rules, and all AEs are evaluated unblinded by the trial management group as well as the TSC according to standard definitions as outlined in online supplementary table 6. In case of unnatural high AE frequencies as compared with the standard clinical practice, the TSC will evaluate if it is ethically possible to continue the study.

The TSC has reviewed the study protocol, statistical analysis plan and the suitability of the proposed safety data to be collected. No interim analysis is planned for this trial.

ETHICS AND DISSEMINATION

The study is performed according to GCP, International Council for Harmonisation (ICH) guidelines and the Declaration of Helsinki.

The study was registered at <https://clinicaltrials.gov/ct2/show/NCT02386917> on 24 February 2015 and last updated on 19 Jan 2018. Any new protocol modifications will be sent for review by the research ethics committee and will be amended at the clinical trial registry.

Details on safety aspects with the different drugs were thoroughly considered and judged to be acceptable before study start (online supplementary table 7). During the PK investigations, patients will be closely monitored,

assessing pulse and general well-being throughout the day. In addition, the investigation room is equipped with acute medication and necessary equipment to take care of any emergency situation before the hospital on-call team will be in place.

The results will be disseminated to academic and health professional audiences via presentations at conferences and publications in peer-reviewed journals. Participants will be sent a summary of the trial findings when the main article is published, and if appropriate, the results will be communicated to policymakers and commissioners of weight management services through briefing papers summarising the main findings.

Author affiliations

¹The Morbid Obesity Center, Vestfold Hospital Trust, Tønsberg, Norway

²Department of Endocrinology, Morbid Obesity and Preventive Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

³Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway

⁴Department of Transplantation Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway

⁵Drug Metabolism and Pharmacokinetics, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca Gothenburg, Gothenburg, Sweden

⁶Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway

⁷Study Operations, Early Clinical Development, IMED Biotech Unit, AstraZeneca Gothenburg, Gothenburg, Sweden

⁸Faculty of Health and Sports Science, University of Agder, Kristiansand, Norway

⁹Department of Physiology and Pharmacology, Section of Pharmacogenetics, Karolinska Institutet, Stockholm, Sweden

¹⁰Cardiovascular, Renal and Metabolism Translational Medicine Unit, Early Clinical Development, IMED Biotech Unit, AstraZeneca Gothenburg, Gothenburg, Sweden

¹¹Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Contributors JH, AÅ, TBA and CK designed the study, and SA, RS, IR, LKJ, PCA, JKH, ES, MH, A-LE, VK, T-IK and HC contributed substantially to develop the protocol and the current work. JH and AÅ drafted the first version of the manuscript, and all authors revised the work critically for important intellectual content. All authors approved the submitted version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. JH is the principal investigator. ES is the trial statistician.

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Competing interests JH, AÅ, RS, LKJ, JKH, ES, VK, T-IK and HC receive no personal financial benefits from the trial. PCA has received a PhD grant, and IR has received a postdoctoral grant from the study budget. CK, TBA, A-LE, MH and SA are employed by AstraZeneca, and CK, A-LE and MH own shares in AstraZeneca.

Patient consent Obtained.

Ethics approval The protocol (version 2, 5 September 2014) was reviewed and approved by the Regional Committee for Medical and Health Research Ethics on 5 November 2014 (Ref: 2013/2379/REK sørøst A) prior to study start on 18 March 2015. The last version of the study protocol with minor amendment (version 4; 12 August 2015) was approved by the Regional Committee for Medical and Health Research Ethics on 5 November 2014 (Ref: 2013/2379/REK sørøst A).

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Data sharing statement According to individual data protection laws in Norway, we are not allowed to publish or share participant-level datasets. The datasets may be available for audit/inspection in special cases on a need-basis only, after a specific application to the Norwegian data inspectorate.

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