Supplementary table 1. Rationale and evidence gap to fill

A. Drug bioavailability and disposition

The current literature shows that body weight, bariatric surgery and weight loss alter oral bioavailability and disposition of drugs, which accordingly may affect both drug efficacy and safety. This study aims to provide novel information and fill a number of gaps in the current knowledge of this topic:

1. *Body weight*. The CYP3A4 metabolizing capacity in individuals with obesity is reduced,[20] but it is unclear what body size measure (e.g. body mass index, ideal body weight, fat free mass) that best correlate with drug metabolizing activities and what the mechanisms behind the regulation are.[44] It is also unclear to what degree other relevant drug metabolizing enzymes and transporters are affected.
2. *Gastric bypass (GBP)* reduces the total surface area available for drug absorption and bypasses the proximal intestine that is rich in metabolizing enzymes (i.e. mainly CYP3A), absorptive (e.g. OATP1A1) and antiabsorptive transporters (e.g. P-gp). These effects will have opposing effects on oral bioavailability of drugs and affect drugs differently depending on their physiochemical characteristics. Detailed information of the expression of metabolizing enzymes and drug transporter at different sites of the GI-tract is limited.[45] Substrates for CYP3A and/or P-gp will show increased bioavailability [33] but limited data on substrates for other CYP:s and transporters is available. The reduced absorptive surface area will mainly affect drugs with low capacity for passive diffusion over cell membranes, but there is little data on the relative contribution of these opposing effects on bioavailability for drugs.[46]
3. *Very low energy diet (VLED)*. The specific effects of calorie restriction and dietary induced weight loss on local expression of metabolizing enzymes and drug transporters in the GI-tract and liver and the effects on oral bioavailability of different drugs are unknown.
4. *Intestinal adaption*. Previous studies in GBP patients indicate that bypassing the proximal intestine increases the oral bioavailability acutely (weeks). This effect seems to be reduced over time (years).[34] We hypothesise that there may be an adaptive process in the intestine over time; that drug bioavailability relevant protein expression in the distal part of the intestine is upregulated when it becomes “proximal” after surgery. The current project will provide intestinal expression data to further elucidate this by assessing biopsies obtained via gastroscopy 2 years after surgery.
5. *Environment/disease regulation*. The regulation of drug metabolizing enzymes and transporters is affected by a wide range of factors, such as disease state, other drugs, food, and pollutants in the environment.[39-43] This study will provide a clearer mechanistic understanding of the impact of inflammation, metabolic status, gut microbiota peptides/proteins and nucleotides on drug metabolizing enzymes and transporters.

Supplementary table 1 (cont.) Rationale and evidence gap to fill

B. Metabolism, cardiometabolic risk factors and biomarkers

The interplay between metabolically active tissues such as skeletal muscle, visceral fat, subcutaneous fat, liver and the gastrointestinal tract is complex and fine-tuned and yet far from understood. This study is designed to disentangle the short-term (6-weeks) metabolic and pharmacokinetic effects of GBP and a VLED by inducing a similar weight loss in the two groups combined with collection of a high-quality biobank and detailed measurements covering different aspects of cardiometabolic diseases. This study thus aims to provide novel information and fill a number of gaps in the current knowledge of this topic:

1. The mechanisms behind development of obesity and obesity-related co-morbidities and improvements of these conditions after weight loss are still not fully understood. This study will generate data to further elucidate these mechanisms.
2. It is still unclear what are the mechanisms behind the acute and dramatic metabolic improvements seen with bariatric surgery.[9] Especially the relative contributions from weight loss and from surgical procedure, respectively, have been inherently challenging to tease out and most mechanistic studies conducted so far are done in pair-fed rodents.[10] However, the mechanisms after bariatric surgery in rodents differ from humans warranting properly designed human studies. This human study has a design that would allow revealing what mechanisms and metabolic changes that are specifically induced by the surgery procedure and by the weight loss per se.
3. The majority of human data on regulation of metabolic pathways in different tissues have been obtained from cross sectional studies, while few prospective studies have addressed short- and long-term changes in tissue compartments after weight loss. This lack of evidence may partly be explained by practical difficulties to obtain serial biopsies from different tissues (e.g. the GI-tract). This study combines in depth analyses of several tissues after serial sampling (subcutaneous adipose tissue, jejunum, gastric ventricle), after single sampling (liver, visceral adipose tissue, skeletal muscle, ileum) and also analyses of serial samples of plasma/urine/faces to provide novel knowledge on how the regulation differs between tissues over time after weight loss induced by different means.
4. Humans possess a circadian clock regulating cell activity in different biological processes with an endogenous, self-sustained period of about 24h.[47] There are growing evidence that internal body time plays a role in the development of diseases.[48] as well as the effect of treatments. For example, the timing of meals in a weight-loss study influenced how much weight people lost [49] and an increased efficacy and reduced toxicity has been shown with chronotherapy in cancer treatment.[50] Interestingly, bromocriptine, a sympatholytic D2-dopamine agonist approved for the treatment of type 2 diabetes in some countries, seems to work by impacting the internal body time.[51] In this study we will repeatedly assess the internal body time as well as inflammation, gut microbiota/antimicrobial peptides, proteins/peptides, nucleotides, lipids, bile acids and investigate how these measures impact/are impacted by/correlate with e.g. body size measures, weight loss induced by diet or gastric bypass surgery, obesity and obesity-related co-morbidities, measures of cardiometabolic diseases, signaling pathways and PK parameters.
5. The current information about genetic variants associated to the topics of the present protocol is somewhat limited when it comes to the metabolic and body composition aspects. This study is small but may provide some important pilot data in this regard.

Supplementary table 2. Schedule of 24-h pharmacokinetic investigations (PKs) and measurements

Prior to the investigation

- The patients are contacted a few days prior to the investigation day and are asked which drugs they have used since the last visit to confirm that no potential interacting drugs are administered prior to the investigation.
- 20:00 two days before the PK investigation. From this time patients are not allowed to drink caffeine-containing beverages.
  22:00 the day before the PK-investigation patients start to fast. Only water is allowed after this time.

On the investigation day

- 07:30 patients meet at the laboratory with a mid-stream morning spot urine and feces sample collected maximum 24-hr before the visit. Physical examination including vital signs, blood pressure, heart rate, EKG, weight, waist- and hip-circumference and bioimpedance and AE-reporting are performed. Medical history, concomitant drugs and doses are noted in the CRF. Pregnancy test for fertile women is performed.
  A peripheral venous catheter is inserted and the following blood samples are drawn: standard clinical chemistry and hematology, baseline samples for study specific analyses as well as baseline cocktail sample, cytokines, 4-β-OH cholesterol and blood samples for epi- and genotyping (selected visits). In addition, a subcutaneous adipose tissue samples is obtained during the first hour.

- 08:00 first blood sample for internal body time and insulin assessment are drawn followed by administration of caffeine (100 mg).
- 08:15 the second fasting insulin sample is drawn.
- 08:30 urine sampling where all urine voiding is collected into a bottle is started.
- 09:00 the rest of the drug cocktail is administered with water. Losartan tablets (2x12.5 mg), omeprazole enterotablets (1x20 mg), digoxin tablet (0.5 mg), midazolam oral syrup (1.5 mg) and rosuvastatin tablet (20 mg).
- Blood samples for analysis of cocktail drugs are drawn at the following time-points: before (0 hours) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 4.25, 4.5, 5, 5.5, 6, 8, 10, 12, 23 and 24 hours following administration. Actual times (24-hour clock) of cocktail administration as well as blood samplings are registered.
- 11:00 caffeine free breakfast is served.
- 12:00 blood sample for internal body time assessment is drawn.
- 13:00 intravenous midazolam (1.0 mg) is administered by a slow infusion over at least 2 minutes.
- 16:00 blood sample for internal body time assessment is drawn.
- 17:00 the last urine is collected (for losartan drug: metabolite measurement).

The cocktail drugs are analysed in the following samples: caffeine and omeprazole (12:00 sample), digoxin, midazolam and rosuvastatin (in a selection from all blood samples obtained during the 24-hr) and losartan (8-hr urine).

Supplementary table 3. Sampling and storage procedures of biological material

The *blood samples* during the PK investigations for drug analysis are drawn in pre-chilled K_2-EDTA vacutainer tubes (no gel or plastic beads) on ice, centrifuged for 10 minutes at 4°C (1800 g), plasma decanted into Cryovials (in 3 parallels, 5 parallels for the 12:00 sample) and frozen within 1 hour at -70°C.

The blood sample (K_2-EDTA) drawn for DNA purification and subsequent genotyping are stored at -70°C.

The blood samples for 4ß-hydroxycholesterol (and comparable endogenous biomarkers) and internal body time determinations are drawn in pre-chilled K_2-EDTA vacutainer tubes (no gel or plastic beads), centrifuged for 10 minutes at 20°C (2200 g) and plasma decanted into Cryovials (in 3 parallels) and frozen immediately at -70°C. Assessment of internal body time will be performed by assessment of at least one steroid and at least one amino acid, e.g. cortisol and tryptophan.

The blood samples (K_2-EDTA) for biobanking and determination of cytokines will be drawn in pre-chilled vacutainers on ice centrifuged for 10 minutes at 4°C (1800 g) and plasma decanted into Cryovials (at least 3 parallels, approximately 1 mL each, for each sample) and frozen immediately at -70°C.

Biopsies are collected in accordance with the SAP (*Appendix 1*) at the time of surgery and at additional time points for some of the tissue biopsies. Biopsies are obtained from gastric ventricle, jejunum, ileum, liver, skeletal muscle, visceral and subcutaneous adipose tissue. All biopsies are snap frozen in liquid nitrogen and stored at -70°C within 2 hours.

Faeces are sampled at all scheduled visits (*Appendix 1*) in specific containers. Five spoons (supplied with the kit) of faeces will be placed in the container and the sealed container will be stored at - 70°C within 48 hours after faeces were collected.

Urine will be sampled at all scheduled visits (*Appendix 1*). The patients are asked to collect morning urine prior to coming to the visit. The urine sample is decanted into two 10 mL tubes and frozen immediately at -70 °C. At the PK investigations urine is also collected during the whole day (8:30-17:00) in a container. The total volume of urine collected is noted and three vials are frozen immediately at -70 °C. One urine sample will be analysed for losartan and two urine samples are stored in the biobank.

Supplementary table 4. List of possible candidate genes/genetic variants/genetic regions relevant for drug transport/metabolism, obesity and metabolic traits

Next-generation sequencing will be applied to capture different types of genetic variation relevant for the probe drug PK, obesity, obesity-related diseases and cardiometabolic status. In this project, we will focus on genes linked to drug metabolism, obesity and obesity-related diseases by adding different filtering strategies.
Drug transport and drug metabolism: CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2B6, CYP3A4, CYP3A5, CYP2D6, UGT1A1, OATP1B1, OATP1B3, OATP2B1, BCRP, MDR1, MRP1, MRP2, MRP4, OCT1, OAT2, NTCP, MATE1, PEPT1.

Obesity and/or BMI: FTO (rs9939609), INSIG2 (rs7566605), PFKP (rs6602024), CTNNBL1 (rs6013029), FDFT1 (rs7001819), MC4R (rs17782313), TMEM18 (rs6548238), SH2B1/ATP2A1 (rs7498665), KCTD15 (rs11084753), NEGR1 (rs2568958), GNPDA2 (rs10938397), MTCH2 (rs10838738), BDNF/LIN7C/LGR4 (rs925946), SEC16B/RASAL2 (rs10913469), FAIM2/BCDIN3D (rs7138803), ETV5/SFRS10/DGKG (rs7647305), NPC1 (rs1805081), MAF (rs1424233), PTER (rs10508503), PRL (rs4712652), RBJ/ADCY3/POMC (rs713586), GPRC5B/IQCK (rs12444979), MAP2K5/LBXCOR1 (rs2241423), QPCTL/GIPR (rs2287019), TNNI3K (rs1514175), SLC39A8 (rs13107325), FLJ35779/HMGCR (rs2112347), LRRN6C (rs10968576), TMEM160/ZC3H4 (rs3810291), FANCL (rs887912), CADM2 (rs13078807), PRKD1 (11847697), LRP1B (rs2890652), PTBP2 (rs1555543), MTIF3/GTF3A (rs4771122), ZNF608 (rs4836133), RPL27A/TUB (rs4929949), NUDT3 (rs206936), NRXN3 (rs10150332), TFAP2B (rs987237), TNKS/MSRA (rs17150703), SDCCAG8 (rs12145833), KCNMA1 (rs2116830), OLFM4 (rs9568856) and HOX5B (rs9299), MC4R (rs12970134), TFAP2B (rs987237), MSRA (rs545854), NRXN3 (rs10146997), LYPLAL1 (rs2605100), RSPO3 (rs9491696), VEGFA (rs6905288), TBX15/WARS2 (rs984222), NFE2L3 (rs1055144), GRB14 (rs10195252), DNM3/PIGC (rs1011731), ITPR2/SSPN (rs7183149), LY86 (rs1294421), HOXC13 (rs1443512), ADAMTS9 (rs6795735), ZNRF3/KREMEN1 (rs4823006), NISCH/STAB1 (rs6784615), MCHR1 (rs133072) and CPEB4 (rs6861681).

Type 2 Diabetes and metabolic traits: PROX1 (rs340874), NOTCH2 (rs10923931), GRB14 (rs3923113), BCL11A (rs243021), RBMS1 (rs7593730), GCKR (rs780094), IRS1 (rs2943641), THADA (rs7578597), ST6GAL1 (rs16861329), ADCY5 (rs11708067), ADAMTS9 (rs4607103), IGF2BP2 (rs4402960), PPARG (rs1801282), WFS1 (rs1801214), ZBED3 (rs4457053), CDKAL1 (rs7754840), KLF14 (rs972283), DGKB (rs972283), GCK (rs4607517), JAZF1 (rs864745), TP53INP1 (rs896854), SLC30A8 (rs13266634), TLE4 (rs13292136), PTPRD (rs17584499), CDKN2A/B (rs10811661), VPS26A (rs1802295), CDC123 (rs12779790), HHEX (rs1111875), TCF7L2 (rs7903146), ARAP1 (rs1552224), HMGA2 (rs1531343), MTNR1B (rs10830963), KCNQ1 (rs2237892, rs231362), KCNJ11 (rs5219), HNF1A (rs7957197), TSPAN8 (rs7961581), SPRY2 (rs1359730), AP3S2 (rs2028299), HMG20A (rs7178572), C2CD4A (rs11071657), ZFAND6 (rs11634397), PRC1 (rs8042680), FTO (rs8050136, rs9939609), SRR (rs391300), HNF1B (rs757210), HNF4A (rs4812829) and DUSP9 (rs5945326).

Supplementary table 5. PROMs Questionnaires to be completed at baseline, 6-week, one-year and two-year follow-up.

Generic Health Related Quality of Life (HRQoL)

Short Form-36 Health Survey (SF-36)

Generic HRQL was assessed using validated Norwegian versions of the Short Form-36 Health Survey (SF-36), 4-week recall, version 2.0 (Vestfold Hospital Trust).[60, 61] The forms were analysed with certified scoring software (QualityMetric Health OutcomesTM Scoring Software 4.0/4.5). Scores for each of the 8 domains and summary scores for physical and mental health were calculated and norm-adjusted using the US 98 population norms to facilitate comparison.

Obesity specific HRQOL

IWQOL-Lite

The IWQOL-Lite is a 31-item measure of weight-related quality of life. There are five domain scores (Physical Function, Self-Esteem, Sexual Life, Public Distress and Work) and a total score. Scores for all domains and total score range from 0-100, with lower scores indicating greater impairment.[62]

Obesity and Weight-Loss Quality of Life (OWLQOL)

The validated obesity specific OWLQOL measures feelings and emotions resulting from being obese and trying to lose weight. [20] The instrument consists of 17 statements rated from zero (“not at all”) to six (“a very great deal) on a seven-point scale. The 17 items form a sum scale ranging from 0-102, with higher scores indicating better emotional HRQOL. As proposed by the authors the scoring syntax converts the scale to 0-100. [63, 64]

Weight-related Symptom Measure (WRSM)

The validated obesity specific WRSM measures 20 symptoms commonly related to being overweight or obese, including foot problems, joint pain, sensitivity to cold, shortness of breath, etc. using two different sets of items. The first set assesses whether or not a patient is experiencing specific symptoms, and the second set rates the level of the distress of the symptoms with values from zero (“not at all”) to six (“bothers a very great deal”). The first set creates an additive scale summing symptoms from 0-20, while the second forms a symptom distress scale ranging from 0-120. [63, 64]

Hospital Anxiety and Depression Scale (HADS)

The validated generic HADS measures symptoms of anxiety and depression using 14 items scored from 0-3.[65] It is decomposed into two domains measuring depression (HADS-D) and anxiety (HADS-A), both consisting of seven items yielding a score from 0-21. Norwegian normative data are available.[66]

Three Factor Eating Questionnaire –R 21 (TFEQ-R21)

The validated generic TFEQ-R21 measures eating behaviour and has been validated for use in individuals with obesity.[67, 68] It consists of 21 items comprising three domain scores; (1) uncontrolled eating; assessing the tendency to lose control over eating when feeling hungry or when exposed to external stimuli, (2) cognitive restraint; assessing the conscious restriction of food intake to control body weight or body shape, and (3) emotional eating; assessing overeating related to negative mood states. The domain scores were transformed to 0-100 scales to facilitate comparison; a higher score indicates more uncontrolled, restraint, or emotional eating.

Supplementary table 6. Adverse and serious adverse events defined

Adverse events (AE) include all medical occurrences during a treatment of study subject with the drug cocktail in the present study. Abnormalities in laboratory values are also included. Changes in laboratory values are only considered to be adverse events if they are judged to be clinically significant, e.g. if some action or intervention is required. If abnormal laboratory values are result of pathology for which there is an overall diagnosis (e.g. increased creatinine in renal failure), the diagnostic term should be reported as the adverse event.

A serious adverse event (SAE) includes every occurrence that at any time point during the study run.

1. Results in death
2. Is life threatening
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability/incapacity
5. Is a congenital anomaly/birth defect
6. Important medical events that may require intervention to prevent one of 1 to 5 above or may expose the subject to danger, even though the event is not immediately life threatening or fatal, or does not result in hospitalization.

Each SAE that is at least possibly related to a study drug is to be classified by the principal investigator as expected or unexpected.

A SAE that is at least possibly related to study drug, and unexpected, is defined as a suspected unexpected serious adverse reaction (SUSAR). It is “expected” if it is already known from earlier studies or is mentioned in available summary of product characteristics (SmPC).

Supplementary table 7. Drug cocktail-safety aspects

Caffeine:	100 mg caffeine is given, which is approximately equivalent to one cup of coffee and is not considered to have any safety issues. Caffeine is not expected to give any side effects but people who never drink coffee, cola or tea can in rare cases experience headache, nausea or palpitations when ingesting caffeine.
Losartan:	Losartan is an angiotensin II-antagonist with antihypertensive effect and the normal therapeutic dose is 50 mg once daily. Half this dose, 25 mg, will be administered in the drug cocktail. The most common side effect of losartan is dizziness, which can occur in about 2% of the patients.
Omeprazole:	Omeprazole is a proton pump inhibitor used for the treatment of acid related gastrointestinal diseases. The therapeutic dose is 20-40 mg once daily and in the present drug cocktail 20 mg is included. In therapeutic doses rare cases of headache, nausea, constipation or diarrhoea can be seen.
Midazolam:	The total dose of 2.5 mg midazolam used in the present study (1.5 mg oral and an additional 1.0 mg iv four hours later) is the lowest dose used clinically when administered for its relaxing effect in adjunction to invasive procedures. No special precautions need to be considered in obese patients and it has previously been used in combination with the other cocktail drugs.
Digoxin:	A single dose of 0.5 mg is considered safe. A simulation of a single dose of 0.5 mg in an obese patient, using RightDose (www.lapk.org), resulted in maximal peak concentrations less than 0.65 µg/L and a trough concentration of 0.9 µg/L and is considered safe.
Rosuvastatin:	Statins are generally well tolerated. The most common adverse effects are myopathy and liver toxicity, however, these usually come after multiple dosing and a single dose of 20 mg has not previously been associated with significant adverse effects.

Interactions between the different components of the cocktail: There is no published information about any relevant pharmacokinetic interactions between the drugs in the present drug cocktail.