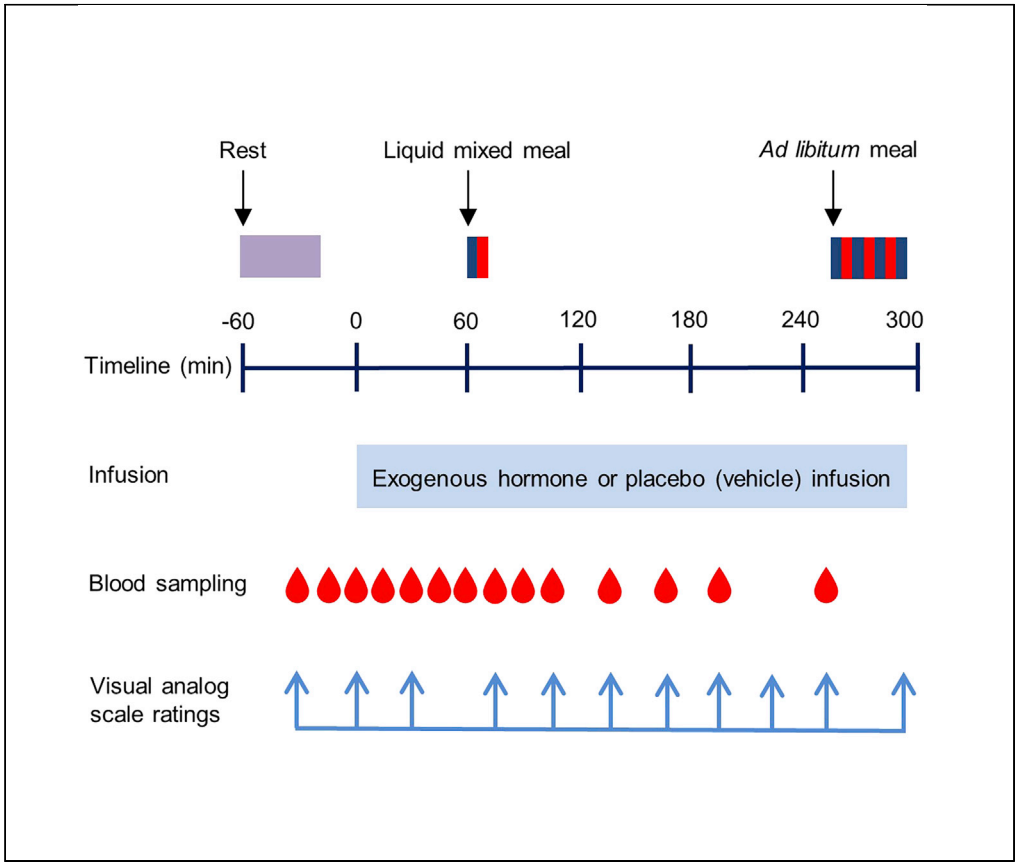
STAR Protocols

CellPress
OPEN ACCESS

Protocol

# Protocol for assessing the effects of exogenous hormone administration on human postprandial glucose metabolism, appetite sensations, and food intake




Here, we present a protocol for a randomized, double-blind, placebo-controlled, crossover trial to evaluate the effects of a continuous intravenous infusion of a native liver-derived hormone, liver-expressed antimicrobial peptide 2 (LEAP2), on postprandial glucose metabolism, appetite and satiety sensations, and *ad libitum* food intake in humans. We describe the preparation of the exogenous hormone administration and participants. We then detail the liquid mixed meal, *ad libitum* meal test, and blood sampling procedures for assessing postprandial glucose metabolism and food intake.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Christoffer A. Hagemann, Lærke S. Gasbjerg, Mikkel B. Christensen, Filip K. Knop

christoffer.andersen.hagemann@regionh.dk (C.A.H.)
filip.krag.knop.01@regionh.dk (F.K.K.)

## Highlights

A randomized, double-blind, placebo-controlled, crossover trial

Evaluation of postprandial glucose metabolism and *ad libitum* food intake in humans

Steps for intravenous administration of native hormones and blood collection

Preparation and serving of a liquid mixed meal and a standardized *ad libitum* meal

Hagemann et al., STAR Protocols 4, 102070
March 17, 2023 
https://doi.org/10.1016/j.xpro.2023.102070

STAR Protocols

CellPress
OPEN ACCESS

Protocol

# Protocol for assessing the effects of exogenous hormone administration on human postprandial glucose metabolism, appetite sensations, and food intake

Christoffer A. Hagemann,[1,2,8,*] Lærke S. Gasbjerg,[1,3] Mikkel B. Christensen,[1,4,5] and Filip K. Knop[1,4,6,7,9,*]

[1]Center for Clinical Metabolic Research, Copenhagen University Hospital Herlev and Gentofte, 2900 Hellerup, Denmark

[2]Gubra, 2970 Hørsholm, Denmark

[3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

[4]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

[5]Department of Clinical Pharmacology, Copenhagen University Hospital Bispebjerg and Frederiksberg, 2400 Copenhagen, Denmark

[6]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

[7]Steno Diabetes Center Copenhagen, Herlev, Denmark

[8]Technical contact

[9]Lead contact

*Correspondence: christoffer.andersen.hagemann@regionh.dk (C.A.H.), filip.krag.knop.01@regionh.dk (F.K.K.)
https://doi.org/10.1016/j.xpro.2023.102070

## SUMMARY

**Here, we present a protocol for a randomized, double-blind, placebo-controlled, crossover trial to evaluate the effects of a continuous intravenous infusion of a native liver-derived hormone, liver-expressed antimicrobial peptide 2 (LEAP2), on postprandial glucose metabolism, appetite and satiety sensations, and *ad libitum* food intake in humans. We describe the preparation of the exogenous hormone administration and participants. We then detail the liquid mixed meal, *ad libitum* meal test, and blood sampling procedures for assessing postprandial glucose metabolism and food intake.**

**For complete details on the use and execution of this protocol, please refer to Hagemann et al. (2022).[1]**

## BEFORE YOU BEGIN

The protocol described here, was used to investigate the effects of liver-expressed antimicrobial peptide 2 (LEAP2) on postprandial glucose metabolism, appetite and satiety sensations, and *ad libitum* food intake in healthy men. We have previously used similarly designed protocols to investigate the effects of other exogenously administered hormones on similar outcome measures.[2,3]

1. Calculate population size using the desired formula for sample size calculation.

   *Note:* The sample size calculation should be based on the chosen significance level and power. A with-in subject standard deviation and minimally relevant difference of the primary endpoint (change in food intake during an *ad libitum* meal assessed by total caloric intake and caloric intake per kg body weight) can be obtained from a previous study analyzing the reproducibility of a single-course *ad libitum* meal test in healthy men.[4]

2. Determine the infusion rate of the continuous intravenous infusion of the exogenous hormone.

   *Note:* It is suggested that the infusion rate of the exogenous hormone is based on data from previous non-clinical studies and, as a minimum, prior experience with peptides of similar length and pharmacokinetic profile. In this case, the infusion rate of LEAP2 targeted a 2–3-fold higher steady state plasma concentration of the native hormone than the endogenous levels in humans.

   *Note:* LEAP2 was administrated at an infusion rate of 25 pmol/kg/min (115 ng/kg/min) resulting in supraphysiological plasma concentrations, i.e., a ~2.6-fold higher plasma concentration of LEAP2 than during placebo infusions.

3. Order the specific peptide, i.e., exogenous hormone, in a quality meeting the requirements for approval from the regulatory authorities.

   *Note:* The exogenous peptide should be confirmed to be identical to the endogenous peptide investigated by the manufacturer, e.g., by high-performance liquid chromatography and mass spectrometry, amino acid sequencing, and/or elemental analysis.

4. Dissolve the peptide in the desired solvent according to the requirements of the regulatory authorities.

   *Note:* LEAP2 was dissolved in saline (9 mg/mL) containing 0.5% human albumin, sterile filtrated, and tested for sterility and endotoxins by the Capital Region Pharmacy of Denmark and dispensed into vials stored at −20°C until use.

5. Recruit participants according to specific inclusion and exclusion criteria.

   *Note:* In this case, inclusion and exclusion criteria favor lean or obese but otherwise healthy men. The inclusion criteria can be adjusted according to the study population of interest. If practically feasible, broad inclusion and exclusion criteria are recommended. Importantly, a larger population size is required if including a more heterogenous population due to a proportionate increase in data variability.

   a. Inclusion criteria for lean men: Caucasian men aged 18–60 years with a body mass index of 20–30 $kg/m^2$.
   b. Inclusion criteria for obese men: Caucasian men aged 18–60 years with a body mass index of 30–60 $kg/m^2$.
   c. Exclusion criteria: Anemia, presence of hepatobiliary and/or gastrointestinal disorder and/or plasma liver enzymes (alanine or aspartate aminotransferases) above two times the normal range, serum creatinine above the normal range and/or albuminuria, regular tobacco smoking and/or use of other nicotine-containing products, allergy or intolerance to ingredients included in the standardized meals, any physical or psychological condition or ongoing medication that the investigators evaluate would interfere with trial participation, and hemoglobin $A_{1c}$ >48 mmol/mol [6.5%] and/or first-degree relatives with diabetes (lean men) or hemoglobin $A_{1c}$ >58 mmol/mol [7.5%], and/or type 2 diabetes requiring medical treatment (obese men).

   *Note:* The recruitment of obese subjects is in our experience far more challenging than recruiting lean individuals. While not always feasible, it is preferable to include gender and age-matched groups to allow for more reliable between-groups comparisons.

   *Note:* Since the total blood loss may amount to a maximum of 450–500 mL (corresponding to approximately a whole blood donation) for both experimental days in total, depending on the

permission from relevant regulatory authorities, only participants with normal hemoglobin should be included. All participants may also be offered iron supplementation (standard iron tablets) after completion of the experimental days.

*Note:* Biochemical markers of liver and kidney disease are measured to exclude any diverse effects from altered clearance of the infused hormone due to hepatic and/or renal insufficiency. Participants with gastrointestinal disorders should be excluded to decrease the inter-individual variability in the postprandial glucose and hormone response. Regular tobacco smoking and nicotine intake are avoided since tobacco smoking and nicotine are known to affect glucose and lipid metabolism possibly due to an increase in plasma catecholamines.[5]

6. Screen participants according to inclusion and exclusion criteria.

⚠ CRITICAL: Screening of a potential participant should only be performed after procuring oral and written informed consent from each person.

*Note:* The screening visit is preceded by a 10-hour fast (water and medications excluded). Measure the potential study participant's height and body weight as well as blood pressure and heart rate after 5 min of rest. Record medication and medical history. Collect blood samples for plasma/serum analyses (creatinine, electrolytes ($Na^+$ and $K^+$), ALAT, ASAT, alkaline phosphatase, bilirubin, albumin, hemoglobin, plasma glucose, and glycated hemoglobin $A_{1c}$) and a urine sample for analysis of albumin-creatinine ratio. In the present protocol, the total blood loss at the screening visit will amount to approximately 20 mL and approximately 10 mL of urine will be sampled. If the investigator finds the participant eligible for the study based on the screening visit, and the participant wishes to be included in the study, the experimental days will be scheduled.

*Note:* Blood and urine samples obtained at the screening visit will be analyzed immediately, and any excess blood/urine will be disposed of.

7. Generate a prespecified randomization list.

⚠ CRITICAL: To ensure double-blinding, the randomization list should be generated by an unblinded staff member not otherwise involved in the study.

*Note:* Consider including a specific block size depending on the required population size. A block size of four is suggested when including 20 participants. The randomization list with the chosen block size can be generated from www.sealedenvelope.com.

### Institutional permissions

The study was performed in accordance with the Declaration of Helsinki (7th revision, 2013) and approved by the Scientific-Ethical Committee of the Capital Region of Denmark and the Danish Data Protection Agency. The study was registered at ClinicalTrials.gov (registration number NCT04621409).

Any experiments on humans will need to acquire permission from relevant regulatory authorities.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Chemicals, peptides, and recombinant proteins | | |
| Saline (9 mg/mL) | Fresenius Kabi | N/A |
| Human albumin (5%) | CSL Behring | Cat# 22,203 |

(*Continued on next page*)

| *Continued* | | |
|---|---|---|
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
| LEAP2 peptide | Biosynth (previously named Vivitide) | N/A |
| Acetaminophen (paracetamol effervescent 500 mg tablets) | GSK Consumer Healthcare | N/A |
| Experimental models: Organisms/strains | | |
| Human subjects (lean or obese healthy men aged 18–60 years) | N/A | N/A |
| Software and algorithms | | |
| FLIR tools program | FLIR Systems | N/A |
| Randomization list | N/A | www.sealedenvelope.com |
| Other | | |
| Indirect calorimetry (Vyntus CPX Canopy) | Vyaire Medical | N/A |
| Thermal imaging camera | FLIR Systems | Cat# A655sc |
| YSI 2900 Biochemistry Analyzer | YSI Inc. | N/A |
| Nutridrink (energy content; 1,010 kJ, 29.7 g carbohydrate, 9.6 g protein and 9.3 g fat per 100 mL) | Nutricia | Cat# 579,760 |
| Standardized meal, e.g., pasta Bolognese (energy content: 565 kJ, 15.0 g carbohydrate, 5.3 g protein, and 5.6 g fat per 100 g) | N/A | N/A |

## STEP-BY-STEP METHOD DETAILS

### Preparation of participants preceding each experimental day

Timing: 48 h prior to each experimental day

*Note:* Careful instructions to participants ensure filled glycogen stores and similar macronutrient balance on both experimental days.

1. Instruct participants to avoid strenuous physical exercise, excessive eating, and alcohol consumption and to keep a food diary for 48 h prior to each experimental day.

   *Note:* The food diary is collected to control for intermittent fasting and/or excessive eating.

2. Instruct participants to consume a standardized meal the evening before experimental days (between 6 and 8 PM) and remain fasted until the experimental day the next morning (equaling approximately 12 h of fasting including any liquids and/or medications).

   *Note:* If possible, the standardized meal should be the same as used in the *ad libitum* meal test and can be provided at the screening visit if kept frozen until used by the participant.

   *Note:* Avoid planning experimental days on Mondays since weekend activities often differ from activities during weekdays in general.

### Experimental days – Day 1 and 2

Timing: 6–7 h each day

The two experimental days are performed in the exact same fashion except for intravenous infusion of native hormone on one day and placebo (vehicle) on the other day in randomized order. The experimental days are performed minimum one week apart. See the graphical abstract for an overview of the clinical study design.

*Note:* During study days, exposure to food advertising from television programs, literature, and other sources should be avoided since food advertising can potentially prime eating behavior.[6]

3. Prepare the infusion according to the randomization scheme after noting the participant's body weight upon arrival in the morning.

⚠ CRITICAL: To ensure double-blinding, the infusions should be prepared to be identically appearing by an unblinded staff member not otherwise involved in the study, and preparing the two infusions must take approximately the same time, i.e., for the sake of the blinding.

*Note:* LEAP2 was diluted to a total volume of 500 mL in saline (9 mg/mL) containing 0.5% human serum albumin. Placebo infusions consisted of 500 mL saline with 0.5% human serum albumin.

4. Insert an intravenous catheter in a cubital vein in each arm of the participant, one for blood sampling and one for infusion of hormone/placebo.

*Note:* The forearm for blood sampling should be kept warm (45°C) to arterialize the venous blood. This can be done by wrapping the hand/forearm in a heating pad or by using a heated box.

5. Collect baseline blood samples and perform procedures for other baseline data (i.e., visual analog scale ratings, indirect calorimetry, collection of urine samples, thermal imaging camera as mentioned in the following sections) according to the chosen sampling scheme and specific instructions for each analytical method included.

*Note:* Blood-based biomarkers are measured from blood samples collected in specific tubes (pre-chilled on ice in some cases) according to the chosen biomarker analyses. Most tubes should immediately be cooled on ice (plain tubes with serum clot activator should be left at room temperature for approximately 20 min to coagulate), centrifuged, pipetted to storage tubes (plasma/serum), and stored at preferably −80°C until analysis. It is suggested to at least measure plasma concentrations of the administrated hormone as well as hormones involved in glucose metabolism such as insulin (and C-peptide) and glucagon. If oral acetaminophen administration is used as an indirect marker of the gastric emptying rate following the liquid mixed meal, plasma concentrations of acetaminophen should be measured (please refer to step 8 for a detailed method description).

*Optional:* Plasma glucose can be measured bedside by the glucose oxidase method (YSI 2900 Biochemistry Analyzer) from blood collected in fluoride heparin-coated tubes and centrifuged immediately for 30 s.

*Note:* Sensations of hunger, satiety, prospective food consumption, fullness, nausea, comfort, and thirst are rated on 100 mm visual analog scales. The reproducibility of this method has previously been validated in healthy men[7] and is often used in studies assessing appetite and satiety sensations.

*Optional:* Measurements of resting energy expenditure and respiratory quotient by indirect calorimetry, protein turnover by renal urea excretion, and/or brown adipose tissue thermogenesis using a thermal imaging camera may be implemented in the study design and sampling scheme. For complete details on the use of these methods, please refer to.[1] For thermal imaging, the room temperature should be similar on all experimental days and registered.

Ideally, a climatic chamber with a constant temperature should be used, but similar room temperatures can also be obtained by standard air conditioning.

6. Start the intravenous infusion of either the exogenous hormone or placebo at time point 0 min.

   *Optional:* It is suggested to take a small sample from the infusion bag before initiating the infusion and after the infusion is terminated, which may be used to measure the concentration of the infused hormone in specific cases, i.e., assessment of stability, randomization issues, etc.

7. Collect blood samples and perform the chosen additional analytical procedures (visual analog scale ratings, indirect calorimetry, collection of urine samples, thermal imaging camera, etc.) throughout the experimental day according to the chosen sampling scheme and specific instructions for each analytical method included.

   *Note:* See notes associated with step number 5 for a detailed description of suggested analytical methods to include.

   *Note:* Blood samples may be collected at various time points. In this case, blood samples were collected at time points −35, −10, 0, 15, 30, 45, 60, 75, 90, 105, 135, 165, 195 and 255 min (for all blood-based analyses) as well as at time points 120, 150, 180 and 225 min (for analysis of plasma glucose only) using a syringe.

   ⚠ CRITICAL: After each blood sampling it is important to infuse approximately 2 mL of saline into the intravenous catheter for blood sampling to avoid blood clotting in the catheter. Just prior to collecting the next blood sample, it is crucial to discard the first ml of blood to avoid dilution of the subsequent blood sample.

8. Prepare and give a liquid mixed meal to the participant approximately 60–90 min after the start of the infusion (time point 60–90 min) depending on the length of the chosen fasting period.

   *Note:* The liquid mixed meal should be consumed evenly throughout 5 min.

   *Optional:* Admix 1,000–1,500 mg acetaminophen dissolved in 50–100 mL water to the liquid mixed meal for assessment of postprandial plasma concentrations of acetaminophen as an indirect marker of the gastric emptying rate, as previously validated.[8,9]

   *Note:* We suggest adjusting the size (energy content) of the liquid mixed meal to the body weight of each participant measured on the first experimental day, e.g., an energy content of 2,400 kJ for an 80 kg person (2.97 mL/kg body weight) when applying the suggested standardized liquid meal (1,010 kJ, 29.7 g carbohydrate, 9.6 g protein and 9.3 g fat per 100 mL). If feasible, the meal volume should be adjusted to lean body mass only, particularly in cohorts with large variations in body composition between included subjects.

9. Serve a standardized *ad libitum* meal together with 400–500 mL of water approximately 200 min after giving the liquid mixed meal (time point 260–290 min depending on the time point for serving the liquid mixed meal).
   a. Instruct the participant to eat as much as they can until they feel comfortably full.

   *Note:* The *ad libitum* meal should be consumed within a maximum of 30 min.

   b. Note the weight of the meal before it is served.

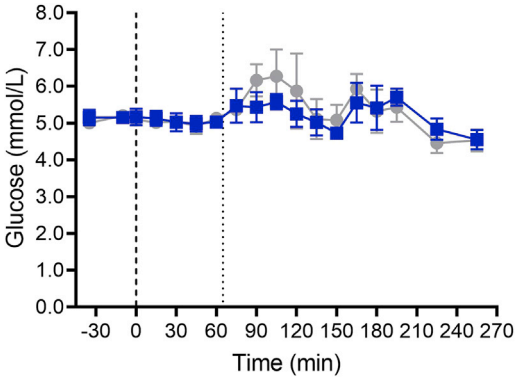



**Figure 1. Plasma glucose concentrations before and after a liquid mixed meal test in three healthy, young men**

The bold and thin dotted lines indicate infusion start (0 min) and liquid mixed meal test start (65 min), respectively. Blue square symbols, LEAP2 infusion; gray round symbols, placebo infusion. Data are presented as mean ± standard error of the mean and originate from Hagemann et al.[1]

⚠ CRITICAL: Serve the *ad libitum* meal in undisturbed surroundings without distraction from mobile phones, other persons, etc. Furthermore, to reduce any psychological and physiological factors affecting food intake,[10] verbal instructions for the meal test, the room used for eating, and heating time of the meal and the bowl should be similar in the two experimental days.

*Note:* It is suggested to instruct the participant to eat directly from the bowl containing approximately 2,000–2,500 g of the standardized *ad libitum* meal to ensure that no participant is close to finishing the meal.

10. Discontinue the infusion of exogenous hormone or placebo when the participant feels comfortable full marking the end of the *ad libitum* meal (at latest, time point 290–320 min depending on the time point for serving the a*d libitum* meal).
    a. Note the time spent eating, the weight of the meal after eating, water intake during the meal, and the total infusion volume during the experimental day.
    b. Calculate the total caloric intake and caloric intake per kg body weight.

## EXPECTED OUTCOMES

In addition to investigating the effects of an exogenous hormone on postprandial glucose metabolism and *ad libitum* food intake, blood-based analyses may provide insights into the potential interaction with other hormones and factors involved in appetite regulation and/or glucose metabolism or biomarkers of lipolysis. Effects of the administrated exogenous hormone on gastric emptying, appetite-related sensations, resting energy expenditure, utilization of metabolic substrates, and/or brown adipose tissue thermogenesis are other suggested outcomes.

Figure 1 illustrates a postprandial plasma glucose response in three healthy men during exogenous LEAP2 and placebo infusions. In the original study,[1] differences in baseline (calculated as a mean of time points −35, −10, and 0 min) and peak values of plasma glucose as well as differences in area under the curve for the entire period (0–255 min), the postprandial period (60–255 min), and the fasting period (0–60 min) between LEAP2 and placebo infusions were tested by Student's paired *t* test. The same statistical analyses were applied to other included plasma measurements.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Comparison of normally distributed data between the paired experimental days may be carried out by Student's paired *t* test or repeated-measures analysis of variance. A two-sided $P$ value $< 0.05$ is usually considered statistically significant.

## LIMITATIONS

This protocol is designed to investigate the effects of exogenous hormone administration in humans and is not designed to answer mechanistic effects of an exogenous hormone in humans.

Since only one dose of an exogenous hormone is used, this protocol does not investigate the dose-response relationship. However, it is possible to expand the protocol to include several doses (one dose per additional experimental day) for comparison against the placebo arm if dose-response relationship is needed.

## TROUBLESHOOTING

### Problem 1

Compliance of participants (related to steps 1 and 2).

### Potential solution

Compliance of participants to the suggested pre-experimental preparations may be difficult to achieve. It is crucial for the experimental day that the participants are fasted and have consumed the standardized meal the evening before. In case of doubt regarding whether the participant is fasting, it is suggested to measure fasting plasma glucose before proceeding with the experimental day. The food diary may also help with clarification of any pre-experimental preparations. Furthermore, frequent contact with participants by telephone or email prior to experimental days is recommended for increasing study compliance.

### Problem 2

Unable to reach a steady state condition during indirect calorimetry (related to steps 5 and 7).

### Potential solution

Estimation of resting energy expenditure and respiratory quotient by indirect calorimetry depends on participants' ability to rest. Steady state is usually defined as a coefficient of variation <10% for $VO_2$ and $CO_2$ over at least 5 min and may be difficult to obtain due to the many other study procedures included in this protocol and, therefore, limited test time. To improve participants' ability to reach a steady state condition, it is recommended that participants are awake and in a supine position from 5 min prior to each test. Furthermore, participants should rest in a bed for 30 min before the first test and throughout the experimental days, and indirect calorimetry should be performed in undisturbed surroundings.

### Problem 3

Urine sampling (related to steps 5, 7, and 9).

### Potential solution

Urine samples may be difficult to collect during experimental days due to the many other study procedures included in this protocol and, therefore, limited sampling time. If feasible, urine sampling may be performed bedside in the experimental room in undisturbed surroundings using a urine container as it is time-consuming and complicated for the participant to move longer distances during the intravenous infusion. If urine samples are not collected during experimental days participants must be offered the chance to urinate before the *ad libitum* meal test as the test might be affected by a strong urge to urinate.

### Problem 4

Change in the settings surrounding the *ad libitum* meal test (related to step 9).

### Potential solution

Identical settings surrounding the *ad libitum* meal test on the paired experimental days are critical when measuring *ad libitum* food intake. If circumstances regarding, e.g., the room or bowl used for

eating are changed, it is recommended that the change applies for both experimental days for the individual participant due to the paired design of the study.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Filip K. Knop (filip.krag.knop.01@regionh.dk).

### Materials availability

This protocol did not generate new unique materials.

### Data and code availability

All data reported in the original study are available from the lead contact on request. This protocol did not generate code.

## ACKNOWLEDGMENTS

None.

## AUTHOR CONTRIBUTIONS

Conceptualization, C.A.H., F.K.K.; methodology, C.A.H., L.S.G., M.B.C., F.K.K.; writing – original draft, C.A.H.; writing – review & editing, C.A.H., L.S.G., M.B.C., F.K.K.; supervision, L.S.G., M.B.C., F.K.K.

## DECLARATION OF INTERESTS

F.K.K. has served on scientific advisory panels and/or been part of speaker's bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MedImmune, MSD/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi, ShouTi, Zealand Pharma, and Zucara. L.S.G. has been in speaker's bureau for Eli Lilly.

## REFERENCES

1. Hagemann, C.A., Jensen, M.S., Holm, S., Gasbjerg, L.S., Byberg, S., Skov-Jeppesen, K., Hartmann, B., Holst, J.J., Dela, F., Vilsbøll, T., et al. (2022). LEAP2 reduces postprandial glucose excursions and ad libitum food intake in healthy men. Cell Rep. Med. *3*, 100582. https://doi.org/10.1016/J.XCRM.2022.100582.

2. Bergmann, N.C., Gasbjerg, L.S., Heimbürger, S.M., Krogh, L.S.L., Dela, F., Hartmann, B., Holst, J.J., Jessen, L., Christensen, M.B., Vilsbøll, T., et al. (2020). No acute effects of exogenous glucose-dependent insulinotropic polypeptide on energy intake, appetite, or energy expenditure when added to treatment with a long-acting glucagon-like peptide 1 receptor agonist in men with type 2 diabetes. Diabetes Care *43*, 588–596. https://doi.org/10.2337/DC19-0578.

3. Stensen, S., Gasbjerg, L.S., Krogh, L.L., Skov-Jeppesen, K., Sparre-Ulrich, A.H., Jensen, M.H., Dela, F., Hartmann, B., Vilsbøll, T., Holst, J.J., et al. (2021). Effects of endogenous GIP in patients with type 2 diabetes. Eur. J. Endocrinol. *185*, 33–45. https://doi.org/10.1530/EJE-21-0135.

4. Gregersen, N.T., Flint, A., Bitz, C., Blundell, J.E., Raben, A., and Astrup, A. (2008). Reproducibility and power of ad libitum energy intake assessed by repeated single meals. Am. J. Clin. Nutr. *87*, 1277–1281. https://doi.org/10.1093/ajcn/87.5.1277.

5. Tweed, J.O., Hsia, S.H., Lutfy, K., and Friedman, T.C. (2012). The endocrine effects of nicotine and cigarette smoke. Trends Endocrinol. Metab. *23*, 334–342. https://doi.org/10.1016/J.TEM.2012.03.006.

6. Harris, J.L., Bargh, J.A., and Brownell, K.D. (2009). Priming effects of television food advertising on eating behavior. Health Psychol. *28*, 404–413. https://doi.org/10.1037/a0014399.

7. Flint, A., Raben, A., Blundell, J.E., and Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int. J. Obes. Relat. Metab. Disord. *24*, 38–48. https://doi.org/10.1038/sj.ijo.0801083.

8. Willems, M., Quartero, A.O., and Numans, M.E. (2001). How useful is paracetamol absorption as a marker of gastric emptying? A systematic literature study. Dig. Dis. Sci. *46*, 2256–2262. https://doi.org/10.1023/A:1011935603893.

9. Glerup, H., Bluhme, H., Villadsen, G.E., Rasmussen, K., Ejskjaer, N., and Dahlerup, J.F. (2007). Gastric emptying: a comparison of three methods. Scand. J. Gastroenterol. *42*, 1182–1186. https://doi.org/10.1080/00365520701370922.

10. Stoeckel, L.E., Birch, L.L., Heatherton, T., Mann, T., Hunter, C., Czajkowski, S., Onken, L., Berger, P.K., and Savage, C.R. (2017). Psychological and neural contributions to appetite self-regulation. Obesity *25*, S17–S25. https://doi.org/10.1002/oby.21789.