Elucidating tissue-specific glucose partitioning and organ perfusion in response to incretin-mediated insulin secretion using total-body PET scan

(The "Incretin PET" study)

Study protocol

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1. Background

Glucose homeostasis ensures the maintenance of blood glucose levels within a narrow range, providing a stable energy supply to tissues and organs (1). This regulation is achieved through a complex interplay of hormones, predominantly the plasma glucose-lowering hormone insulin and the plasma glucose-elevating hormone glucagon secreted by pancreatic beta and alpha cells, respectively (1). Upon secretion, insulin facilitates glucose uptake into insulin-sensitive tissues, primarily in skeletal muscle, adipose tissue, and the liver (1). Further, insulin promotes glycogenesis, the conversion of glucose to glycogen, particularly in the liver and skeletal muscle, and inhibits gluconeogenesis, the hepatic endogenous glucose production from non-carbohydrate substrates, preventing excessive glucose release into the bloodstream (1). In contrast to insulin, glucagon acts to raise blood glucose levels during fasting or metabolic stress. Glucagon stimulates glycogenolysis, the breakdown of glycogen into glucose, and enhances gluconeogenesis (1).

Using the cutting-edge technology of a Siemens Vision Quadra total-body PET-CT scanner with a four times axial field of view (i.e., 106 cm) and higher temporal and spatial resolution compared to conventional PET-CT scanners, it is possible to assess dynamic and simultaneously whole-body assessment of glucose uptake and organ perfusion across tissues and organs within the field of view. Positron emission tomography (PET) is a functional imaging technique that uses radioactively labelled tracers to visualise physiology including perfusion and substrate uptake. When the radioactive isotope undergoes decay, the emitted positron travels a few millimeters in the tissue and collides with an electron. Consequently, the two particles annihilate, and two photons are emitted in opposite directions. The photons are registered by detectors and are then transformed into images. In the present project, we use the PET tracers, ^{18}F-fluoro-deoxy-glucose (FDG), a glucose analogue that serves as a proxy for glucose uptake, and $H_2{}^{15}O$, a freely diffusible water molecule that provides a quantitative measure of tissue blood flow. We combine PET with computed tomography (CT) to ensure an anatomical foundation for the PET scan. CT scans use X-rays to create detailed cross-sectional images of the various tissues and organs of the human body (2).

The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) play a key role in postprandial glucose homeostasis by enhancing insulin secretion in response to nutrient intake (1). Both hormones are secreted from enteroendocrine cells in the gut (2). These gut hormones are responsible for the increased insulin secretion in response to an oral glucose load compared to an intravenous glucose infusion (i.e., the incretin effect) (3).

The insulin-mediated regulation of plasma glucose levels is a well-characterized process, yet the specific partitioning of glucose into different organs and tissues, including the role of tissue perfusion, remains unknown. While it is established that insulin facilitates glucose uptake primarily into skeletal muscle, adipose tissue, and the liver, the relative contributions of glucose across these and a variety of other tissues and organs, and the extent to which this is influenced by insulin-mediated changes in perfusion, require further elucidation. Insulin seems to increase perfusion of primarily skeletal muscle (4) and adipose tissue (5), ensuring delivery of both

insulin and glucose to these insulin-sensitive organs. Insulin-induced augmentation of organ perfusion is also suggested in the myocardium (6) and may extend to additional organs. Whether tissue perfusion differs in individuals with type 2 diabetes compared to healthy individuals is currently unknown.

Applying dynamic whole-body PET-based techniques in combination with incretin hormone-stimulation of endogenous insulin secretion, we can describe the physiological role of endogenous insulin on glucose partitioning and organ perfusion during a simulated postprandial physiological state of high plasma glucose and incretin hormone concentrations.

2. Overall objective and aim of the project

The overall objective is to investigate insulin-induced glucose uptake in and organ perfusion of skeletal muscle as well as other organs and tissues of interest in the postprandial state.

The project is divided into two sub-studies. The primary aim of **sub-study 1** is to evaluate the metabolic rate of FDG (MR_{FDG}), as a surrogate for glucose, in skeletal muscle facilitated by endogenous insulin during a simulated postprandial physiological state of elevated plasma glucose and incretin hormone concentrations. Further, in **sub-study 1**, we aim to explore the insulin-mediated FDG uptake across a variety of additional tissues and organs including the brain, myocardium, liver, bones, intestines, pancreas, spleen, and adipose tissue. The primary aim of **sub-study 2** is to evaluate the perfusion of skeletal muscle facilitated by endogenous insulin in both healthy individuals and individuals with type 2 diabetes. Also, in **sub-study 2**, we aim to assess perfusion across a variety of additional tissues and organs including adipose tissue, liver, jejunum, the heart, and kidneys in both healthy individuals and individuals with type 2 diabetes.

Finally, as one of the first projects to apply these interventions and measurements, the project will generate reference FDG and $H_2{}^{15}O$ total-body PET data to be used in future research.

3. Hypotheses

We hypothesise that MR_{FDG} in and perfusion of skeletal muscle is larger in the postprandial state with high endogenous insulin concentrations, respectively, compared to placebo. In addition, we hypothesise that during infusions of GIP and GLP-1, to stimulate endogenous insulin production and simulate the postprandial state, respectively, compared to placebo:

- percentage FDG uptake, mean and max standardised uptake values (SUV) of FDG, FDG influx (K_i), and intracellular phosphorylation rate (k_3) in skeletal muscle, adipose tissue and the liver are increased
- percentage and absolute perfusion (ml/min/g tissue) of skeletal muscle, adipose tissue and liver are increased

Finally, we hypothesise that during hormone infusions, skeletal muscle exhibits the highest percentage FDG uptake and perfusion compared to additional organs assessed in the field of view.

4. Study designs

	Interventions	Participants	Study procedures
Sub-study 1	Infusion with GIP, GLP-1 or saline Infusion with glucose	12 individuals with BMI 20.0-26.9 kg/m^2	FDG-PET-CT scans
Sub-study 2	Infusion with GIP or GLP-1 Infusion with glucose	12 healthy individuals and 12 age, sex and BMI-matched individuals with type 2 diabetes	$H_2{}^{15}O$-PET-CT scans

In **sub-study 1**, twelve individuals with BMI 20.0-26.9 kg/m^2 are included in this randomised, placebo-controlled, single-blind, crossover study involving three experimental days for each participant: one with intravenous infusion of GIP, one with intravenous infusion of GLP-1, and one with intravenous infusion of saline (placebo). On experimental days, participants will undergo a total-body PET-CT scan preceded by an intravenous bolus administration of FDG.

In **sub-study 2**, twelve healthy individuals and twelve age, sex and BMI-matched individuals with type 2 diabetes are included in this randomised, placebo-controlled, single-blind, crossover study involving two experimental days for each participant: one with intravenous infusion of saline followed by GIP and one with intravenous infusion of saline followed by GLP-1. On experimental days, participants will undergo a total-body PET-CT scan with recordings of perfusion using six bolus infusions of $H_2{}^{15}O$.

4.1. Endpoints

The primary endpoint is MR_{FDG} in in skeletal muscle (**sub-study 1**) and perfusion of skeletal muscle (**sub-study 2**) assessed with total-body PET-CT scans during infusion of GIP and GLP-1, respectively, compared with saline (placebo).

Secondary endpoints include:

- Percentage FDG uptake in skeletal muscle, adipose tissue, and liver
- MR_{FDG} in liver and adipose tissue
- Mean and maximum SUV in skeletal muscle, liver, and adipose tissue
- Intracellular phosphorylation rate (k_3) in skeletal muscle, liver, and adipose tissue
- K_i in skeletal muscle, liver, and adipose tissue
- Circulating concentrations of glucose, GIP, and GLP-1

- Circulating concentrations of insulin, C-peptide, and glucagon
- Perfusion of adipose tissue and liver

Exploratory endpoints include:

- Percentage FDG uptake in brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- MR_{FDG} in the brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- Mean and maximum SUV in the brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- k_3 in the brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- K_i in the brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- Time activity curves of FDG concentrations in skeletal muscle, liver, adipose tissue, brain, myocardium, kidneys, bones, intestines, pancreas, and spleen
- Perfusion of bone, jejunum and kidneys
- Time activity curves of perfusion of bone, adipose tissue, liver, jejunum and kidneys

For both **sub-study 1** and **sub-study 2**, all endpoints will be evaluated between infusion with GIP and GLP-1, respectively, and saline. For **sub-study 2**all endpoints will also be compared between healthy individuals and individuals with type 2 diabetes.

4.2. Participant eligibility criteria

In **sub-study 1**, twelve individuals will be included according to the following inclusion and exclusion criteria.

Inclusion criteria:

- Age 23-50 years at the time of inclusion
- BMI 20.0-26.9 kg/m^2
- Informed consent

Exclusion criteria:

- Anaemia (haemoglobin below normal range)
- Alanine aminotransferase (ALT) >2 times normal values or present hepatobiliary and/or gastrointestinal disorder(s)
- Kidney disease (serum creatinine above normal range)
- Previous gastric or intestinal resection (excluding appendectomy and cholecystectomy) and/or any major intra-abdominal surgery (including bariatric surgery)
- Glycated haemoglobin (HbA1c) ≥42 mmol/mol and/or type 1 diabetes or type 2 diabetes requiring medical treatment

- Regular tobacco smoking or use of other nicotine-containing products
- Claustrophobia
- Initiation of special diets, lifestyle changes and/or weight loss >5% of total body weight within three months prior to or during the study period
- Breastfeeding or pregnancy or desire to become pregnant during the study period
- Any ongoing medication as well as physical or psychological condition that the investigator evaluates would interfere with trial participation
- Inability to speak and/or read Danish

In **sub-study 2**, twelve individuals with type 2 diabetes will be included according to the following inclusion and exclusion criteria.

Inclusion criteria:

- Age 23-60 years at the time of inclusion
- Diagnosed with type 2 diabetes (HbA1c >53 mmol/mol) at least 3 months prior to enrolment
- Treatment of diabetes with metformin
- Informed consent

Exclusion criteria:

- Anaemia (haemoglobin below normal range)
- Use of glucose-lowering drugs other than metformin
- Alanine aminotransferase (ALT) >2 times normal values or present hepatobiliary and/or gastrointestinal disorder(s)
- Kidney disease (creatinine above normal range)
- Previous gastric or intestinal resection (excluding appendectomy and cholecystectomy) and/or any major intra-abdominal surgery (including bariatric surgery)
- Chronic obstructive pulmonary disease
- Regular tobacco smoking or use of other nicotine-containing products
- Claustrophobia
- Breastfeeding or pregnancy or desire to become pregnant during the study period
- Inability to speak and/or read Danish
- Any ongoing medication as well as physical or psychological condition that the investigator evaluates would interfere with trial participation

Also, in **sub-study 2**, twelve age, sex and BMI-matched healthy individuals will be included according to the following inclusion and exclusion criteria.

Inclusion criteria:

- Age 23-64 years at the time of inclusion
- Glycated haemoglobin (HbA1c) <42 mmol/mol
- Informed consent

Exclusion criteria are similar to those for the healthy individuals.

4.3. Adjournment of trials

Decision of withdrawal from the study can be made by the investigator group or the participant in question at any time. The study is terminated for each participant in case they wish to withdraw from the protocol, or in case exceptional circumstances make it impossible to complete the study. In case of premature termination, the participants will be informed about the decision and underlying reason(s).

4.4. Recruitment of participants

Participants will be recruited among individuals who have previously participated in trials at Center for Clinical Metabolic Research, Copenhagen University Hospital – Herlev and Gentofte, and Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital – Rigshospitalet, and at that time accepted to be contacted again regarding other research projects. These individuals will be reached through their preferred method of communication (phone or email) as per their consent. Also, participants will be recruited by advertising in newspapers, in general practice, and/or on the internet (such as www.forskningsnu.dk, www.trialtree.dk, www.gentoftehospital.dk). For this, the enclosed materials for recruitment will be used. Furthermore, a short video will be used to recruit people with type 2 diabetes in sub study 2 (see attached). This video will be shared on social media platforms. Comment sections and the option to tag individuals will be disabled to prevent unintended collection of personal information. Potential participants who reach out after advertising are contacted by telephone or email.4.5. Study procedures

All study procedures are carried out at the Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital – Rigshospitalet. **Sub-study 1** consists of a screening visit, a dual energy X-ray absorption (DXA) scan, and three experimental days with intravenous infusion of GIP, GLP-1 and saline (placebo), respectively. The experimental days are performed with a minimum washout period of one week between visits. **Sub-study 2** consists of a screening visit, a dual energy X-ray absorption (DXA) scan and two experimental days with intravenous infusion of saline followed by GIP or GLP-1, respectively. The experimental days are interposed by a minimum washout period of 7 days.

4.6. Screening and DXA scan

The screening visit is similar across all three sub-studies. Height, body weight, blood pressure, heart rate, and waist and hip circumferences are measured. Medication and medical history are recorded, and blood samples are drawn and analysed for creatinine, electrolytes (Na^+ and K^+),

liver enzyme (ALT, AST), amylase, alkaline phosphatase, bilirubin, albumin, cholesterols, triglycerides, haemoglobin, thrombocytes, hsCRP, TSH, plasma glucose, and HbA_{1C}. The total amount of blood drawn at the screening visit is approximately 20 ml. 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, experimental days are planned. Body composition of each participant is assessed using a DXA scan within four weeks from the screening date.

4.7. Experimental days

An overview of the experimental days of **sub-study 1** is shown in Figure 1. The procedures of the three experimental days are identical except for the infusion of GIP, GLP-1 or saline (placebo). Prior to each experimental day, participants are instructed to avoid strenuous physical exercise, excessive eating, intermittent fasting, and alcohol consumption for 72 hours. Participants will keep a food diary for 72 hours prior to each experimental day to document their intake of food and fluids and identify any instances of intermittent fasting or excessive eating (see Appendix 1). Participants are instructed to adhere to similar diets for 24 hours prior to the three experimental days. Experimental days are carried out in the afternoon. On each experimental day, the participants are instructed to consume a liquid breakfast (Resource®, Nestlé Health Science, Vevey, Switzerland) followed by a minimum of six hours of fasting excluding a maximum of 500 ml water ingested evenly over the six-hour fasting period. Participants must avoid physically strenuous means of transportation to get to the research facility on experimental days. Upon arrival at our department, female participants deliver a urine sample for evaluation of urine hCG before initiating experimental procedures and the test will be destroyed immediately after. In case of a positive urine hCG test, the participant will be excluded from further participation in the study. Thereafter, two intravenous catheters are inserted, one for the collection of blood samples and one for infusion of GIP, GLP-1 or saline (placebo) and glucose. Blood samples are collected throughout the experimental day and will amount to approximately 126 ml per experimental day. At time -30 min, an infusion of GIP (priming dose of 18 pmol/kg body weight/min for 10 minutes followed by steady-state dose of 6 pmol/kg body weight/min), GLP-1 (4.5 pmol/kg bodyweight/min for 10 minutes followed by 1.5 pmol/kg body weight/min) or saline (placebo) (0.9% sodium chloride) is initiated. At time -20 min, a glucose clamp with a target plasma glucose concentration of 6.5-7.5 mmol/l is initiated. Plasma glucose concentration is evaluated every 5 minutes throughout the clamp period. At time -5 min, an ultra-low-dose CT (1.5 mSv) is performed for attenuation correction and anatomical mapping. At time 0 min, a bolus of FDG (0.7 MBq/kg body weight) (~1.5 mSv) is administered followed by 70 min of dynamic PET acquisitions. Infusions of GIP, GLP-1 or saline (placebo) and the glucose infusion will be terminated when the total-body PET scan is completed. Participants are served a soft drink before leaving the facilities.

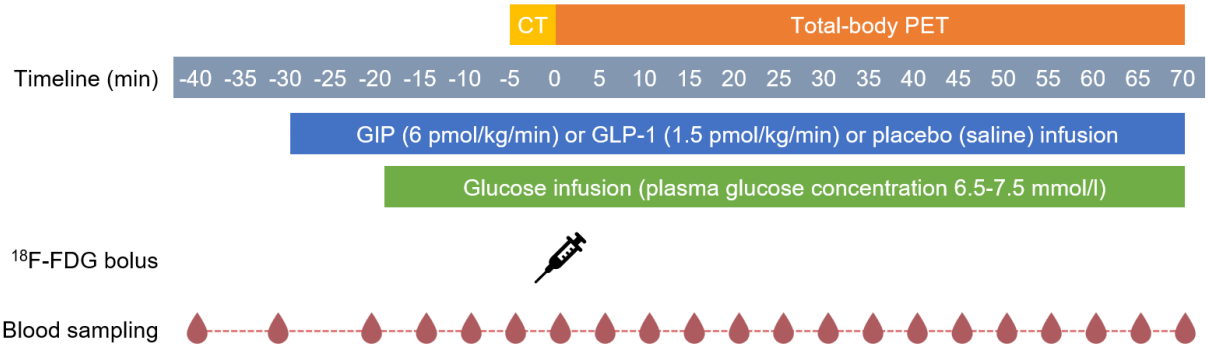

Figure 1. Overview of experimental days of sub-study 1. ^{18}F-FDG, ^{18}F-fluoro-deoxy-glucose; CT, computed tomography; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; PET, positron emission tomography.

An overview of the experimental days of **sub-study 2** is shown in Figure 2. The procedures of the two experimental days are identical except for the infusion of GIP or GLP-1. Instructions to participants, meal standardisation, urinary hCG measurement, catheter placement are similar to sub-study 1.Blood sampling collected throughout the experimental day and will amount to approximately 102 ml per experimental day. A low-dose CT (2.5 mSv) is performed for attenuation correction and anatomical mapping. At time 0 minutes, a glucose clamp with a target plasma glucose concentration of 6.5-7.5 mmol/l for healthy participants and fasting glucose plus 1.5-2.5 mmol/l for participants with type 2 diabetes is initiated. Plasma glucose concentration is evaluated every 5 minutes throughout the clamp period. Also, at time 0 minutes, saline (0.9% sodium chloride) will be infused over 15 minutes. In that period, two bolus infusions of $H_2{}^{15}O$ will be given. After the saline infusion, at time 15 minutes, an infusion of GIP (priming dose of 18 pmol/kg body weight/min for 10 minutes followed by steady-state dose of 6 pmol/kg body weight/min) or GLP-1 (4.5 pmol/kg bodyweight/min for 10 minutes followed by 1.5 pmol/kg body weight/min) is initiated. During infusion of GIP or GLP-1, four bolus infusions of $H_2{}^{15}O$ will be given at intervals of ~10 minutes. Infusions of GIP or GLP-1 and the glucose infusion will be terminated when the total-body PET scan is completed. Participants are served a soft drink before leaving the facilities.

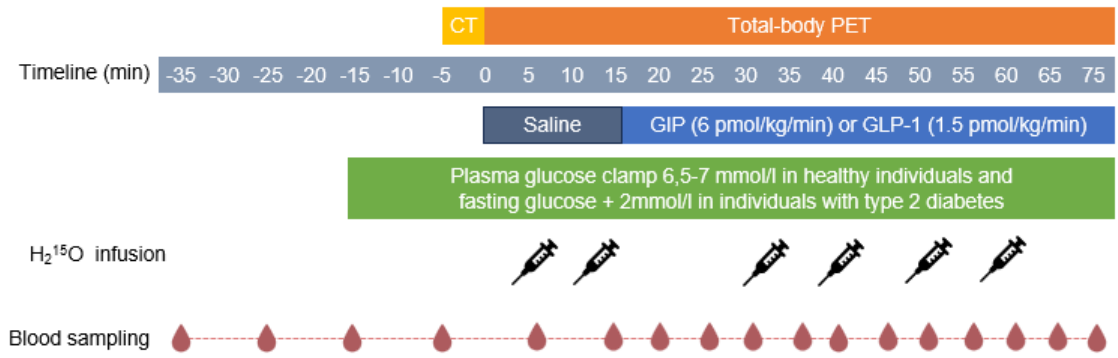

Figure 2. Overview of experimental days sub-study 2. CT, computed tomography; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; $H_2{}^{15}O$, oxygen-15 labelled water; PET, positron emission tomography.

4.8. Blood sampling

For the measurement of plasma glucose, a drop of blood will be applied to glucose test strips and analysed with a bedside glucose analyser. Blood collected in chilled tubes containing EDTA and a specific dipeptidyl peptidase 4 inhibitor will be centrifuged immediately and stored at -20°C or 80°C until analyses of glucagon, GIP, GLP-1, insulin and C-peptide.

4.9. Blinding

Experimental days of all sub-studies will be conducted single-blinded (blinded for the participant but not the investigator) in a randomised order. The PET physicists will remain blinded to the order of infusions until calculations are completed. The randomisation sequence is generated using www.sealedenvolope.com with a block size of three or four.

5. Biobank

Blood sampled at screening visits is analysed immediately, and any excess samples are destroyed. A research biobank will be established to store plasma from the experimental days until analysis. After the project is completed, the research biobank is terminated. The project is expected to be completed by 31st December 2027. From January 2028, excess plasma is stored in another biobank for future research in pseudonymised form for up to 10 years after the completion of the study until destruction, re-analyses, further analyses or new research projects. Excess plasma is stored in accordance with the rules of The Danish Data Protection Agency. Further analyses and/or new research projects including plasma samples from the present study require renewed approval from the Regional Health Research Ethics Committee as well as renewed informed oral and written consent from participants, however a dispensation from informed consent renewal can be obtained from the committee. At all times, and without further explanation, participants can have their samples retracted from the research biobank. The biobanks will be registered and approved by The Danish Data Protection Agency.

6. Informed consent

Prior to any protocol-related procedures, the potential study participant is offered an information visit. Before this visit, the participant receives written information about the project along with the document "*Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt*" and are informed about the possibility of bringing a companion to the information visit. At the information visit, an investigator from the project group explains the purpose, procedure, and possible side effects of the study in undisturbed and confidential surroundings. When someone other than the principal investigator conducts the information sessions, a written delegation agreement is made at the study site between the principal

investigator and the person providing the information. Based on the information visit, if the individual decides to participate, oral and written consent are obtained, and a screening visit is planned. Before signing the consent form, the participant is offered at least 24 hours to reconsider. Should the participant need further time, a follow-up meeting will be scheduled. Participants are informed that they may, at any time, withdraw their informed consent to participate in the study without having consequences for their future treatment. No study-related activities are conducted before the informed consent form has been signed by the participant and investigator.

7. Use of medical records with consent

When written and oral consent is obtained, members of the project group will access the medical record of the participant (through "Sundhedsplatformen") to evaluate the results of screening blood samples, result of DXA-scan, medical history looking for history of anaemia, kidney disease, previous gastric or intestinal resection, type 1 or 2 diabetes, regular tobacco smoking, claustrophobia, pregnancy and ongoing medication. This access is necessary to evaluate whether the possible participant can be enrolled in the study. Furthermore, the oral and written consent allows regulatory authorities to directly access the medical records of the participant to monitor the project.

8. Side effects, risks, and disadvantages for study participants

Intravenous infusion of GLP-1 and GIP are well-established and safe with no observed side effects. As a theoretical complication associated with intravascular catheters (and any other penetration of the skin and blood vessels with sharp/pointed objects), superficial phlebitis should be mentioned. Superficial phlebitis is not dangerous and is treated with antibiotics should any sign of infection be present. The risk of superficial phlebitis is small and minimised by following clinical standards for the insertion of intravenous catheters, including double cleansing of the skin with an ethanol swab and other sterile procedures. Total amount of blood drawn is approximately 90 ml per experimental day and 20 ml per screening, amounting to approximately 400 ml in **sub-study 1** and 300 ml in **sub-study 2**. Only participants with haemoglobin within the normal range are included. Low-dose CT scans and the radioactive tracers will expose participants to a total radiation dose of approximately 9 mSv corresponding to approximately 3 years of background radiation (annual background radiation in Denmark is 3 mSv). A DXA scan will expose the participants to a radiation dose of approximately 0.1 mSv. Theoretically, participation in one of the sub-studies can increase the lifetime risk of cancer from 25% to 25.05% (calculated using, *Appendiks 2 – Retningslinjer om anvendelse af ioniserende stråling i sundhedsvidenskabelige forsøg*, from the Danish National Health Research Ethics Committee).

9. Physical and mental integrity and privacy of study participants

Information about participants in the project and samples stored in the research biobank are protected in accordance with the applicable laws of Denmark including the Data Protection Act and the General Protection Regulation. The protocol will be reported to The Danish Data Protection Agency via the Capital Region of Denmark.

10. Remuneration and reimbursement of costs for study participants

Apart from a general medical examination, the project will not benefit the individual participant. For the inconvenience and the time spent, participants in **sub-study 1 or sub-study 2** will each be remunerated DKK 750 (taxed as B-income) for each completed experimental day. If participants choose to withdraw prematurely from the study, they will be reimbursed according to their time spent on the project.

11. Calculations, statistical analyses and sample size

11.1. Calculations

For all sub-studies, anatomical organ segmentation of relevant organs is completed by an AI algorithm (TotalSegmentor (7)) developed by Siemens and tested by Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital – Rigshospitalet. In **sub-study 1**, simple semi-quantitative uptake measures such as mean and maximum SUVs corrected for injected dose, time and weight of the participant are estimated for each organ. Further, based on the dynamically acquired images, time-activity curves are calculated for each organ and kinetic modelling performed (based on 3-compartment modelling) enabling estimation of absolute glucose uptake including discrimination between uptake (influx rates), MR_{FDG} and intracellular phosphorylation rate. In **sub-study 2**, dynamic PET images are acquired, and time-activity curves are generated for each segmented organ. Perfusion is quantified using a validated one-tissue compartment model specifically developed for $H_2{}^{15}O$-assessed perfusion, which allows estimation of absolute tissue perfusion (ml/min/g tissue) based on the tracer kinetics and arterial input function.

11.2. Baseline characteristics

For all sub-studies, categorical data will be summarized as number and percentage, normally distributed data as mean and standard deviation, and non-normally distributed outcomes as median and quartiles.

11.3. Primary endpoint

For **sub-study 1**, change in MR_{FDG} in skeletal muscle between the three experimental days is evaluated in a linear mixed model with treatment and period as fixed effects and with an unstructured covariance to account for repeated measurements on each study participant and

potential variance heterogeneity between treatments. No test for carry-over effect is performed. Missing data is handled implicitly by maximum likelihood inference in the linear mixed model. For **sub-study 2**, differences in skeletal muscle perfusion between infusions of saline and GIP as well as saline and GLP-1 are evaluated in a linear mixed model similar to that applied in sub-study 1. A two-sided *P* value <0.05 is considered statistically significant.

11.4. Secondary and exploratory endpoints

For all sub-studies, secondary and exploratory endpoints will be analysed similar to the primary endpoint. Also, in sub-study 2, endpoints are compared between healthy individuals and individuals with type 2 diabetes. Substantially skewed outcomes are log-transformed prior to analysis. *P* values from secondary and exploratory endpoints are adjusted separately for multiple testing using the method of Benjamini and Hochberg which controls the false discovery rate. An adjusted *P* value <0.05 is considered statistically significant.

11.5. Sample size calculation

The statistical power of the experiments (1 - β) is set to 80%, corresponding to a type II error rate (β) of 20%, which reflects the probability of failing to reject a false null hypothesis. The sample size of **sub-study 1** was estimated using the power.t.test function from the *pwr* package in R (version 4.3.0). Based on unpublished data from a similar study conducted by our research group evaluating the effects of liver-enriched antimicrobial peptide 2 (LEAP2) using FDG-PET, we assumed a standard deviation of 46% for the change in MR_{FDG} in skeletal muscle. With a sample size of 12 participants, this design allows for the detection of a minimal relevant difference of 41% in MR_{FDG} in skeletal muscle.

In **sub-study 2**, we are confident that standard deviation of the difference between skeletal muscle perfusion during saline and GIP/GLP-1 infusion will not exceed 50%. With a sample size of 12 participants in each group, this allows for the detection of a minimal relevant difference of 44% in perfusion of skeletal muscle.

12. Funding and duality of interest

The initiative to this project is taken by Lærke Smidt Gasbjerg, MD, PhD, and Per Cramon, MD, PhD. All study procedures will take place at Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital – Rigshospitalet. The investigators will not receive any personal financial reimbursement from conducting the study. Expenses for the project are covered by financial grants from Danish Independent Research Council / Sapere Aude Research Leader 2025 (10.46540/5253-00046B: 1.1 MIO DDK) and the Novo Nordisk Foundation (NNF23OC0084114: 800.000 DDK). The Regional Health Research Ethics Committee will be informed if further funding is achieved, and the participant information sheet will be updated accordingly. Funds will be placed in research accounts with Department of Biomedical Sciences, University of Copenhagen, Center for Clinical Metabolic Research, Copenhagen University Hospital – Herlev and Gentofte, or Department of Clinical Physiology

and Nuclear Medicine, Copenhagen University Hospital – Rigshospitalet, all under financial audit of Danish authorities.

13. Significance of the project

The project has the potential to provide detailed and imperative information about physiological glucose metabolism as well as organ perfusion and provide highly requested reference data for future research using this novel whole-body PET-based technology. This area of research can lead to a more holistic understanding of metabolic diseases and may identify previously unknown connections between metabolic processes in different tissues.

13.1. Publication of study results

Data from the studies will be presented in two or more manuscripts for publication in international scientific journals. Results will be published in accordance with the law concerning processing of personal data and publication will be performed both in the case of positive, negative, or inconclusive results.

13.2. Availability of data for the study participants

All study data will be managed in a pseudonymous manner, that is, every study participant will receive a unique trial number used to identify data sheets and tubes. Full name, social security number and trial number will be stored separately. Only the principal investigator and designated investigators will be able to re-assign the trial number to the actual study participant. After the study has been completed, the study participants will receive written information about the study results.

14. Exposition to the Regional Health Research Ethics Committee

The protocol complies with the principles of the Helsinki Declaration (Eighth Revision, 2024) and national guidelines. All participants will receive detailed oral and written information. Oral and written consent is obtained prior to any protocol-related activity. The project group reports no conflicts of interest. All participants will be assigned a trial number and will on data sheets and tubes only appear with a trial number. The full name, social security number and trial number will be stored separately. Excess plasma will be stored in a biobank for future research for up to 10 years from 1st January 2028 in case of any re-analyses or the need for further analyses or new research projects. In the latter case, a new approval by the Regional Health Research Ethics Committee will be required.

Please refer to the section “Side effects, risks, and disadvantages for participants” for a detailed description of risks, side effects, and disadvantages for study participants. In summary, the risks of participating in the present study are minor, and the study group predicts that serious adverse events in response to infusion with the naturally occurring hormones GIP and GLP-1, respectively, are very unlikely, especially since equivalent doses of GIP and GLP-1 have

repeatedly been infused in humans during clinical studies with no report of adverse event. Thus, the study group is convinced that the potential scientific benefits of the study outweigh the hazards and burdens for the study participants. The project will add valuable information to our understanding of glucose metabolism and organ perfusion.

15. Patient compensation

Any unexpected serious adverse events will be reported to the Regional Health Research Ethics Committee. If necessary, participants are covered by the Patient Compensation Association ("*Patienterstatningen*").

16. References

1. Röder P V., Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med*. 2016;48(December 2015):e219. doi: 10.1038/emm.2016.6

2. Jorsal T, Rhee NA, Pedersen J, Wahlgren CD, Mortensen B, Jepsen SL, et al. Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. *Diabetologia*. 2018 Feb;61(2):284–94. doi: 10.1007/s00125-017-4450-9

3. Creutzfeldt W. The incretin concept today. *Diabetologia*. 1979 Feb;16(2):75–85. doi: 10.1007/BF01225454

4. Baron AD. Hemodynamic actions of insulin. *Am J Physiol Metab*. 1994 Aug;267(2):E187–202. doi: 10.1152/ajpendo.1994.267.2.E187

5. Emanuel AL, Meijer RI, Muskiet MHA, Van Raalte DH, Eringa EC, Serné EH. Role of Insulin-Stimulated Adipose Tissue Perfusion in the Development of Whole-Body Insulin Resistance. *Arterioscler Thromb Vasc Biol*. 2017;37(3):411–8. doi: 10.1161/ATVBAHA.116.308670

6. Liu Z. Insulin at physiological concentrations increases microvascular perfusion in human myocardium. *Am J Physiol - Endocrinol Metab*. 2007;293(5):1250–5. doi: 10.1152/ajpendo.00451.2007

7. Wasserthal J, Breit H-C, Meyer MT, Pradella M, Hinck D, Sauter AW, et al. TotalSegmentator: Robust Segmentation of 104 Anatomical Structures in CT images. 2023; doi: 10.5281/zenodo.6802613